Eesti Maaülikool
Estonian University of Life Sciences

POLYPHENOLIC COMPOSITION OF RHUBARB (*RHEUM RHAPONTICUM* L.) AND BLACKCURRANT (*RIBES NIGRUM* L.), ANTIBACTERIAL AND FREE RADICAL SCAVENGING PROPERTIES OF THESE PLANTS IN COMPARISON WITH SOME OTHER FOOD PLANTS

HARILIKU RABARBERI (*RHEUM RHAPONTICUM* L.) JA MUSTA SÕSTRA (*RIBES NIGRUM* L.) POLÜFENOOLNE KOOSTIS, NENDE TAIMEDE ANTIBAKTERIAALSE TOIME JA VABADE RADIKAALIDE SIDUMISE VÕIME VÕRDLUS MÕNEDE TEISTE TOIDUTAIMEDEGA

PIRET RAUDSEPP

A Thesis
for applying for the degree of Doctor of Philosophy in Food Science

Väitekiri
filosoofiadoktori kraadi taotlemiseks toiduteaduse erialal

Tartu 2021

Eesti Maaülikooli doktoritööd

Doctoral Theses of the
Estonian University of Life Sciences

www.emu.ee
Eesti Maaülikool
Estonian University of Life Sciences

POLYPHENOLIC COMPOSITION OF RHUBARB (*RHEUM RHAPONTICUM* L.) AND BLACKCURRANT (*RIBES NIGRUM* L.), ANTIBACTERIAL AND FREE RADICAL SCAVENGING PROPERTIES OF THESE PLANTS IN COMPARISON WITH SOME OTHER FOOD PLANTS

HARILIKU RABARBERI (*RHEUM RHAPONTICUM* L.) JA MUSTA SÕSTRA (*RIBES NIGRUM* L.) POLÜFENOOLNE KOOSTIS, NENDE TAIMEDE ANTIBAKTERIAALSE TOIME JA VABADE RADIKAALIDE SIDUMISE VÕIME VÕRDLUS MÕNEDE TEISTE TOIDUTAIMEDEGA

PIRET RAUDSEPP

A Thesis
for applying for the degree of Doctor of Philosophy in Food Science

Väitekiri
filosoofiadoktori kraadi taotlemiseks toiduteaduse erialal

Tartu 2021

Institute of Veterinary Medicine and Animal Sciences
Estonian University of Life Sciences

According to verdict Nº 3 of October 27, 2020, the Doctoral Committee of Veterinary and Food Science of Estonian University of Life Sciences has accepted the thesis for the defence of the degree of Doctor of Philosophy in Veterinary Medicine and Food Science.

Opponent: **Professor Baoru Yang, PhD**
Food Chemistry and Food Development Unit
Faculty of Science and Engineering
University of Turku, Finland

Supervisors: **Professor Tõnu Püssa, PhD**
Chair of Food Hygiene and Veterinary Public Health
Institute of Veterinary Medicine and Animal Sciences
Estonian University of Life Sciences

Dr. Ave Kikas, PhD
Polli Horticultural Research Centre
Institute of Agricultural and Environmental Sciences
Estonian University of Life Sciences

Defence of the thesis:
International video conference in the BigBlueButton web room at 10.00, December 10, 2021.

The publication of this dissertation is supported by the Estonian University of Life Sciences.

Language editors:
Mrs. Ursula Erik and Ingrid Helvi Williams (English)
Mrs. Urve Ansip (Estonian)

ISSN 2382-7076
ISBN 978-9916-669-11-2 (trükis)
ISBN 978-9916-669-12-9 (pdf)

”Live and learn!”

Ms Inga Paaskivi
In years 1991 to 1994

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The present thesis is based on the following research papers, which are referred to by their Roman numerals (**I-IV** in the text). The papers are reproduced with kind permission of the publishers.

I Püssa, T., **Raudsepp, P.**, Kuzina, K., Raal, A., 2009. Polyphenolic composition of roots and petioles of *Rheum rhaponticum* L. Phytochemical Analysis, 20, 98−103.

II **Raudsepp, P.**, Kaldmäe, H., Kikas, A., Libek, A.-V., Püssa, T., 2010. Nutritional quality of berries and bioactive compounds in the leaves of black currant (*Ribes nigrum* L.) cultivars evaluated in Estonia. Journal of Berry Research, 1, 53−59.

III **Raudsepp, P.,** Anton, D., Roasto, M., Meremäe, K., Pedastsaar, P., Mäesaar, M., Raal, A., Laikoja, K., Püssa, T., 2013. The antioxidative and antimicrobial properties of the blue honeysuckle (*Lonicera caerulea* L.), Siberian rhubarb (*Rheum rhaponticum* L.) and some other plants, compared to ascorbic acid and sodium nitrite. Food Control, 31, 129−135.

IV **Raudsepp, P.,** Koskar, J., Anton, D., Meremäe, K., Kapp, K., Laurson, P., Bleive, U., Kaldmäe, H., Roasto, M., Püssa, T., 2019. Antibacterial and antioxidative properties of different parts of garden rhubarb, blackcurrant, chokeberry and blue honeysuckle. Journal of the Science of Food and Agriculture, 99, 2311−2320.

The contribution of the author Piret Raudsepp (PR) to the papers

I PR performed the HPLC-UV-Vis-MS/MS analyses of the samples and participated in the writing of the manuscript.

II PR performed the sample preparation and HPLC-UV-Vis-MS analyses of the samples, the data collection and analyses, composed the figures and was the main compiler of the manuscript.

III PR participated in the study designing, performed the sample preparation, the HPLC-UV-Vis-MS/MS analyses and data collection, interpreted the results of the HPLC-UV-Vis-MS/MS and spectrophotometric analyses and was the main compiler of the manuscript.

IV PR participated in the study designing, performed the sample preparation, the HPLC-UV-Vis-MS/MS analyses and data collection, interpreted the results of chemical analyses, composed the figures and some of the tables, was the main compiler of the manuscript.

ABBREVIATIONS

AB – antibacterial (properties)

ABTS – 2,2'-azinobis(3-ethylbenzothiazoline)-6-sulfonate

AO – antioxidativity, antioxidative properties

AO% – antioxidative efficiency, the reduced amount of DPPH%

BC – blackcurrant

BPC – base peak chromatogram

CHD – coronary heart disease

CLSI – Clinical and Laboratory Standards Institute, USA

CVD – cardiovascular disease

DPPH – 2,2-diphenyl-1-picrylhydrazyl stable free radical

DST – diagnostic sensitivity test (agar)

DW – dry weight

EC_{50} – 50% of the maximal effective concentration

EFSA – European Food Safety Authority

ESCOP – European Scientific Cooperative on Phytotherapy

EU – European Union

FCR – Folin-Ciocalteu reagent reactivity

FINELI – national food database in Finland

FRAP assay – ferric reducing antioxidant potential measuring assay

FRS – free radical scavenging

FT-NIR – Fourier transform near-infrared spectroscopy

FW – fresh weight

Gram− – Gram-negative bacterial species

Gram+ – Gram-positive bacterial species

HPLC – high performance liquid chromatography

HPLC-MS/MS – high performance liquid chromatograph, coupled with a mass selective detector with an additional fragment ions analyzer

HPLC-UV-MS^2 – high performance liquid chromatograph, coupled with ultraviolet/visible light detector and mass selective detector with an additional fragment ions analyzer

IBA – International Blackcurrant Association

LC/MSD Trap – Agilent 1100 series liquid chromatograph/mass selective detector of ion trap type

LDL – low-density lipoprotein particles

m/z – ion mass to charge ratio,

MIC – minimal inhibitory concentration

MRS broth (Oxoid) – agar, developed by de Man, Rogosa and Sharpe in 1960 for cultivation and enumeration of *Lactobacillus spp*.

MUFAs − monounsaturated fatty acids

NutriData – food composition database, by The National Institute for Health Development, Estonia. ©NutriData food composition database, version 9.0, tka.nutridata.ee, 2019 https://tka.nutridata.ee/en/

NE – niacin equivalents

PCA – principal component analysis

Ph. Eur.– European Pharmacopoeia

PUFAs − polyunsaturated fatty acids

RE – retinol equivalents

RDA – recommended daily allowance of a nutrient

SB – sea buckthorn

SPE – solid phase extraction

SUSMEATPRO – "Sustainable plant ingredients for healthier meat products – proof of concepts"

Syn. – synonym

TAC – total antioxidative capacity

TBARS – thiobarbituric acid reactive substances

TE − tocopherol equivalents

TEAC – trolox equivalent antioxidant capacity

TPC – total polyphenol content, total polyphenols

USDA – U.S. Department of Agriculture

UV-Vis – ultraviolet and visible light

1. INTRODUCTION

Blackcurrant (BC) (*Ribes nigrum* L.) and garden rhubarb (*Rheum rhaponticum* L.) synonym (syn.) *Rheum altaicum* Losinsk. syn. Siberian rhubarb syn. culinary rhubarb are well- known important horticultural plants. In 2017, *Ribes nigrum* L. was cultivated worldwide on 55,616 ha (49,416 tons) (International Blackcurrant Association (IBA), 2017), and in Estonia on 626 ha (479 tons) (Maaeluministeerium, 2018). In 2019, according to the British Growers Association, 25,000 tons of garden rhubarb were grown in England (British Growers Association, 2019). Blackcurrant berries contain anthocyanins, various other polyphenols and organic acids including ascorbic acid; the leaves of blackcurrant are high in polyphenols and aromatic volatile compounds (Dvaranauskaite *et al.*, 2008). Both berries and leaves are used in making energizing beverages (Vagiri, 2014). Rhubarb petioles contain polyphenols, including anthocyanins (Takeoka *et al.*, 2013), but are also high in organic acids and fibre. The extracts of the roots of garden rhubarb have been used to relieve the symptoms of menopause (Heger *et al.*, 2006; Hasper *et al.*, 2009). Both species are regarded as health promoting (Hasper *et al.*, 2009; European Pharmacopoeia, 2019). Additionally, either both species or the polyphenolic compounds contained in them, have been tested for free radical scavenging (Tabart *et al.*, 2006; Jakobek *et al.*, 2007; Milivojević *et al.*, 2010; Chai *et al.*, 2012; Ahmad *et al.*, 2014) and antimicrobial (Kosikowska *et al.*, 2010; Ziad *et al.*, 2011; Abdel-Massih and Abraham, 2014; Nowak *et al.*, 2016) properties.

The species *Ribes nigrum* L. as well as *Rheum rhaponticum* L. comprise multiple cultivars. They are also grown as seedlings of unknown origin. Therefore, the nutritional value and other properties may differ widely within a species (Rumpunen and Henriksen, 1999; Vagiri *et al.*, 2013; Mattila *et al.*, 2016). In addition to cultivar (genotype), soil and weather conditions, maturity, part of the plant and processing technology are all factors that may influence the chemical composition of these or any plants or the products made from them (Zheng *et al.*, 2012; Vagiri *et al.*, 2013; Vagiri *et al.*, 2015). Therefore, we have to bear in mind that the chemical composition of plant material, at the time of analysis, is a reflection of that moment only and the results may be different at another time point (personal communication with Professors Pierluigi Gaboni and Tõnu Püssa). Nevertheless, if we have collected the samples, preserved, prepared and consequently analysed them under similar

conditions, we may compare different plant species in terms of composition and *in vitro* AB or FRS properties in the same assay. In the present thesis, special emphasis was focused on the polyphenolic composition of blackcurrant berries and leaves (**II**) and rhubarb petioles and roots (**I**). The *in vitro* free radical scavenging (FRS) and antibacterial (AB) properties of these plant parts were compared with several other highly valued cultivated or wild berries and fruits (blue honeysuckle, bilberry, chokeberry, sea buckthorn and tomato) in two different experiments (**III**; **IV**). Antioxidativity (AO) and antioxidative (AO) properties are considered in this study mostly as the free radical scavenging capacity.

The present work contributed to the international research project "Sustainable plant ingredients for healthier meat products – proof of concepts" (SUSMEATPRO). The purpose of that project was to find plant materials or side stream materials of plant production that could be used in meat products in order to enhance their healthiness and prolong their shelf life. In the first part of the project, the working groups of different countries estimated the composition and tested the *in vitro* free radical scavenging and antibacterial properties of the selected plant materials. The present study provides an overview of the first phase of the above mentioned project, conducted in Estonia. In the second part, the most promising plant materials were used in meat products, where the FRS properties and total bacterial count were monitored in the duration study (Anton *et al.*, 2019). The sensory properties of the enriched meat products were evaluated separately in every participating country and most thoroughly by the Danish working group (SUSMEATPRO).

2. REVIEW OF THE LITERATURE

2.1. Polyphenols in plants

Over 9000 phenolic compounds have been identified in plants (Cardona *et al.*, 2013). Polyphenols comprise a group of secondary metabolites, which are synthesized in the biosynthetic pathway, starting with the phenyl ring containing amino acids − phenylalanine or tyrosine (Fig. 1) (Tian *et al.*, 2008; Deffieux *et al.*, 2009; Emiliani *et al.*, 2009; Quideau *et al.*, 2011). Polyphenols can be divided into several groups, based on their chemical structure and condensation rate of the rings (Hardman, 2014; Basheer and Kerem, 2015; Papuc *et al.*, 2017). The basic structure of polyphenols consists of one or more phenyl rings, which may also contain heterocyclic ring, containing oxygen, and to which two or more hydroxyl groups, and carboxyl or glycosyl groups are attached (Fig. 2). The latter ones are called glycosides. In plant tissuses, polyphenols exist mainly in glycosylated forms (Cardona *et al.*, 2013). Aglyconic monomeric polyphenols may be condensed into tannins, ellagitannins (based on hydroxybenzoic acids), procyanidins (based on epicatechin and catechin) and into other bigger molecules (Fig. 2).

Figure 1. Phenylalanine (left) and tyrosine (right), the starting substances of polyphenol synthesis

Polyphenols have many functions in plants. For example, anthocyanins – the red-purple coloured pigments serve to attract pollinators and seed spreaders (Kong *et al.*, 2003). They also protect the plant tissues from UV radiation, cold, and pest attack, as do several other polyphenol compounds (Steyn *et al.*, 2002; Winkel-Shirley, 2002; Jeandet *et al.*, 2020).

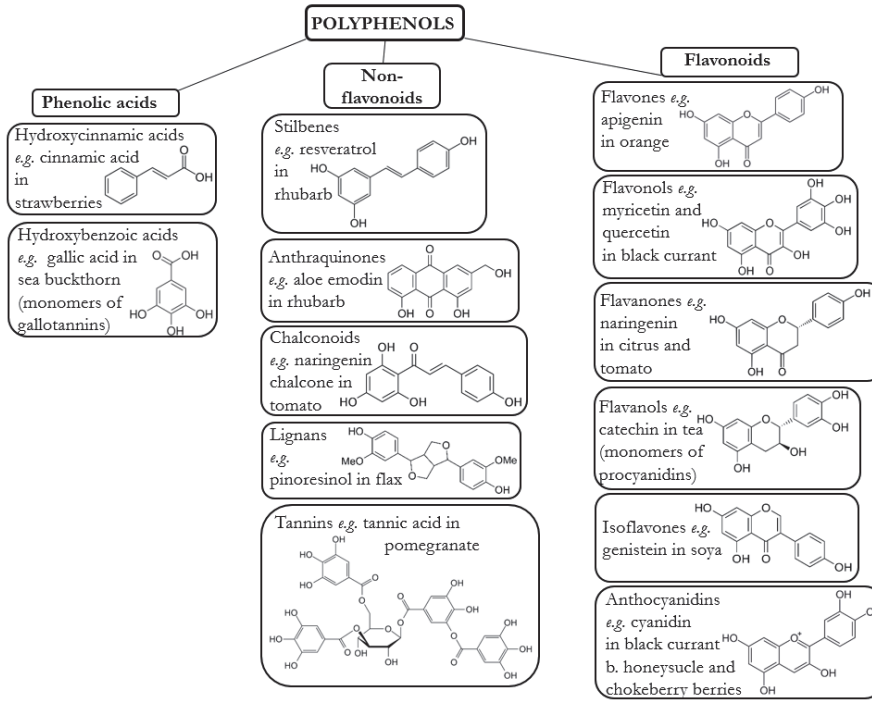


Figure. 2. The classes of polyphenols. The figure is composed based on Hardman, 2014; Basheer and Kerem, 2015; Papuc *et al*., 2017

2.2. Human health benefits of polyphenols

A large number of clinical studies have established that many foodstuffs, containing different polyphenols, especially flavonoids, have anti-oxidative (Zafra-Stone *et al.*, 2007; Zhao *et al.*, 2012; Tonin e*t al.*, 2015), anti-inflammatory (Hardman, 2014), cardioprotective (Wang *et al.*, 2014) and other health beneficial properties (Manach *et al.*, 2005; Tonin *et al.*, 2015; Grosso, 2018). The link between consumption of polyphenol rich foods and the gut-brain axis has been established, as these foods create an advantage for the beneficial microbiota in the human intestine (Cardona *et al.*, 2013; Fraga *et al.*, 2019). Moreover, dried and fresh fruits of bilberry (*Vaccinium myrtillus* L.), blackcurrant (*Ribes nigrum* L.) leaves, and roots of some rhubarb species (*Rheum palmatum* L. and *Rheum officinale* Baillon), and many other plant parts have been incorporated in the European Pharmacopoeia (Ph. Eur.) as herbal drugs (European Pharmacopoeia, 2019). Nevertheless, polyphenols are not the only group of biologically active or health beneficial compounds in plants, they contain also fiber, sulphur compounds, water- and lipid

soluble vitamins and their precursors, organic acids, volatile compounds *etc.* (Manach *et al.*, 2005). And therefore, despite all the available scientific data, showing the health beneficial properties of different polyphenol compounds or foods rich in them, according to the European Food Safety Authority (EFSA) journal (EFSA, 2011), the right to use the health claim on polyphenols is so far granted only to the polyphenols of olive oil (Hohmann *et al.*, 2015). The substantiated claim is "Olive oil polyphenols (standardised by the content of hydroxytyrosol (Fig. 3)) contribute to the protection of blood lipids from oxidative stress" (Commission Regulation, 2012).

In order to substantiate more health claims for other polyphenol rich foods in the future, uniformity of the scientific study design has to be reached, in order to conduct, especially clinical trials, in the way that they can meet the EFSAs requirements, regarding the health claims that can be granted for foods or food components. In addition, the biochemical mechanism of action of polyphenols is still largely unknown, mainly due to the wide variation of the molecules that all classify as polyphenols and due to the great diversity of their natural sources. For example, Kimble *et al.* (2018) concluded in their meta-analysis that anthocyanins, specifically anthocyanidins, reduce the risk of coronary heart disease (CHD) and cardiovascular disease (CVD) mortality. However, they also concluded that further randomized controlled trials on anthocyanin intake and CVD risk factors are needed to support these findings and most importantly, the bioavailability of the polyphenols in general has to be investigated more thoroughly.

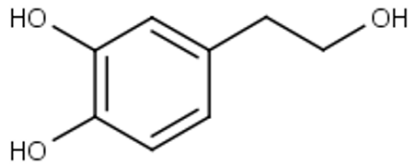


Figure 3. Structure of phenylethanoid − hydroxytyrosol, found in olive oil

2.3. Intake and bioavailability of polyphenols

Polyphenols occur in plants mainly as glycosides (Cardona *et al.*, 2013). The hydrolytic separation of the glycosyl moiety takes place in acidic conditions (through acid-catalyzed hydrolysis), during heating of the plant material or in the course of preservation due to enzymatic hydrolysis. After these processes, elevated amounts of aglycones can be

detected in the sample. After consumption of plant-derived foods or beverages, dietary polyphenols are absorbed in the small intestine or metabolized with the help of the gut microbiota in the large intestine (Cardona *et al.*, 2013; Fraga *et al.*, 2019; Tomás-Barberán and Espín, 2019). There are studies (Manach *et al.*, 2005), showing absorption of both glycosides and aglycones in the intestines, both forms may then be found in the blood or in other tissues (He *et al.*, 2006). Cardona *et al.* (2013) mentioned that the human body recognizes polyphenols as xenobiotics and their bioavailability is therefore relatively low in comparison to micro- and macronutrients. Polyphenols are thus bioavailable only to some extent; their metabolites circulate in the blood as conjugates with glucuronic acid, methyl, or sulphate groups, attached in the liver, and they are eventually excreted in the urine (Almeida *et al.*, 2018).

The average intake of polyphenols from daily diet can be 1,492±665 mg/day (Manach *et al.*, 2005; Taguchi *et al.*, 2015), consumed in the composition of beverages or food. Yet, the concentration of the total polyphenol metabolites (Day *et al.*, 2001; He *et al.*, 2006; Piwowarski *et al.*, 2016; Noh *et al.*, 2017; Almeida *et al.*, 2018) in plasma has been detected only as ~ 80 μmol/l (Manach *et al.*, 2005). This may suggest that there are several possible pathways of polyphenols metabolism in the organism or even that their fate and possible metabolites are yet largely unknown (Manach *et al.*, 2005; Kimble *et al.*, 2018).

2.4. Possible adverse effects of polyphenols

Due to the large variety of polyphenolic compounds, it is not safe to say that all of these compounds are beneficial for human health in elevated doses (Coultate, 2009; Nepomuceno, 2011; Boncler *et al.*, 2017). There are groups of polyphenols *e.g.* isoflavones that have an oestrogenic effect, which may interfere with thyroid hormone synthesis (Mennen *et al.*, 2005) or some that may have a cytotoxic effect *e.g.* kaempferol (Boncler *et al.*, 2017) or may act as prooxidants (Sergediene *et al.*, 1999). The polyphenols that act as free radical scavengers may adversely interfere with cancer treatment (Coultate, 2009; Nepomuceno, 2011). As with all pharmacologically active compounds, the health effect of polyphenols is dose dependent (Sergediene *et al.*, 1999; Mennen *et al.*, 2005). Therefore, consumed in the composition of original- and mixed foods, most polyphenols are generally regarded as safe but in

concentrated form, *e.g.* in food supplements, a proper risk assessment is needed before receiving permission to sell them, and their use is sometimes restricted (Mennen *et al.*, 2005). Therefore, it is not foreseen in the near future that pure polyphenols in extracts or powdered forms will be permitted by the EFSA, to be used in foods as additives, except for anthocyanins as colourants. Within the present thesis, all of the compared plant parts are regarded as food plants, except the root of garden rhubarb. In clinical studies, the extract of rhubarb (*Rheum rhaponticum* L.) root has proved to be safe to use in treatment of perimenopausal symptoms (Hasper *et al.*, 2009), but the effect of the root as a food component for the general population and safe doses are yet unknown.

In the second phase of the SUSMEATPRO project (Anton *et al.*, 2019), a maximum of 2% of plant powders (not concentrated extracts) were used in meat products. It was noticed that even 1% of the garden rhubarb root powder in meat was not organoleptically well liked, which means that the use of this powder in some food matrices cannot exceed this percentage for taste reasons. This finding may give an opportunity to evaluate, in the following studies, the risks, related to the addition of rhubarb root powder to foods in the abovementioned proportions.

2.5. Analytical methods for determination of polyphenols

2.5.1. Extraction methods

Polyphenols are semi-polar compounds, *i.e.* they can be extracted using solvents having heterogeneous properties, starting with water (as in the plant vacuole sap, where the polyphenols are located in plants) and ending with pure lower alcohols. Extraction yield varies due to many factors (Durazzo and Lucarini, 2019). Fig. 4 illustrates the elution order of different polyphenol classes from reversed phase liquid chromatographic column, depending on the mobile phase composition, that generally can be taken into account already when extracting the samples. It has been shown that the highest yield of polyphenols can be obtained in the interval of 20% to 70% of ethanol or other water soluble organic solvents by maceration, followed by filtration, depending on the classes of polyphenols of interest (Lapornik *et al.*, 2005; Rusak *et al.*, 2008). Decoction with cold water that is gradually heated to the hot (boiling) state can be used to obtain food grade extracts. For that

purpose, also the supercritical CO_2 extraction technique could provide valuable input as the extraction is almost solvent free and therefore food grade extracts can be obtained (Renard, 2017; 2018). In addition, percolation and the Soxhlet method can be used, where the solvent is continuously renewed and heated (Bisset and Wichtl, 1994). To facilitate and speed up the extraction, pulsed electric field, microwave-, ultrasound-, or enzymatic treatment, or accelerated pressure can be applied (Renard, 2017; 2018). It is advised to reduce the solvent pH < 3, especially when extracting anthocyanins, to maintain their colour stability (Kylli, 2011).

After solvent extraction, the samples must be centrifuged and filtered to obtain a clear filtrate for spectrophotometric or high performance liquid chromatographic (HPLC) analysis. Sometimes the solid phase extraction (SPE) columns are used to remove saccharides and possible haze before HPLC analysis (Kylli, 2011). On some occasions, the samples are subjected to acidic or enzymatic hydrolysis to retain only the polyphenol aglycones in the samples.

2.5.2. Analytical methods

2.5.2.1. Folin-Ciocalteu method

Many researchers use the Folin-Ciocalteu method to determine total polyphenol content in plant samples. The method is based on the reduction of the Folin-Ciocalteu reagent also called Folins phenol reagent, by phenolic compounds in alkaline solution, where in the course of the reaction a complex is formed that can be quantified at 755−765 nm with a spectrophotometer. The quantitative results are usually expressed in gallic acid equivalents (Blainski *et al.*, 2013).

The problem is, that various other compounds, *e.g.* ascorbic acid, other acids, vitamins, peptides, amino acids, may also have reducing properties in solution and therefore the total amount of polyphenols may be overestimated. Huang *et al.* (2005) and Csepregi *et al.* (2016) suggest therefore using this method instead for the estimation of total FRS capacity. Alternatively, at least the content of ascorbic acid has to be subtracted from the results (Vagiri *et al.*, 2013).

2.5.2.2. Spectrophotometric method

This method is based on the ability of the anthocyanin group, differently from other polyphenols, to absorb light at 500−520 nm in slightly acidic solutions (Lee *et al.*, 2006). The absorbance intensity is linearly correlated with the concentration of anthocyanins and can be measured spectrophotometrically. The problem is that betalaines, cochineal and some artificial dyes like ponceau 4R, carmoisine, amaranth and erythrosine also have absorption maxima at the same wavelengths (Lee *et al.*, 2006, Coultate, 2009). Therefore, by using this method, food fraud (adding artificial colorants or betalaines to obtain reddish-purple colours in foods) cannot be discovered. In addition, the qualitative composition of the anthocyanins cannot be determined with this method.

2.5.2.3. Quantification of condensed tannins

Quantification of condensed tannins is based on the precipitation of tannins from the sample with methylcellulose, with absorbance spectrophotometrically measured at 280 nm before and after the precipitation (Sarneckis *et al.*, 2006). The method enables quantification of condensed tannins in epicatechin equivalents and is used mostly in the wine and cider industry.

2.5.2.4. pH differentiation method

The pH differentiation method for determination of monomeric anthocyanins is based on the pH dependent colour change of monomeric anthocyanins (Fig. 5). Anthocyanins exist at pH 1.0 in the form of the coloured flavylium cation, and at pH 4.5 in the colourless hemiketal form. The difference in the absorbance of these pigments at 520 nm is proportional to pigment concentration. Results are usually expressed on a cyanidin-3-glucoside basis (Lee *et al.*, 2006). The absorbance of the extracts at 700 nm at pH 1.0 and pH 4.5 is subtracted to take into account possible haze in the extract. With this method beetroot betalains and cochineal colour start to interfere with the results, when their concentration in the solution is over 10% (Lee *et al.*, 2006).

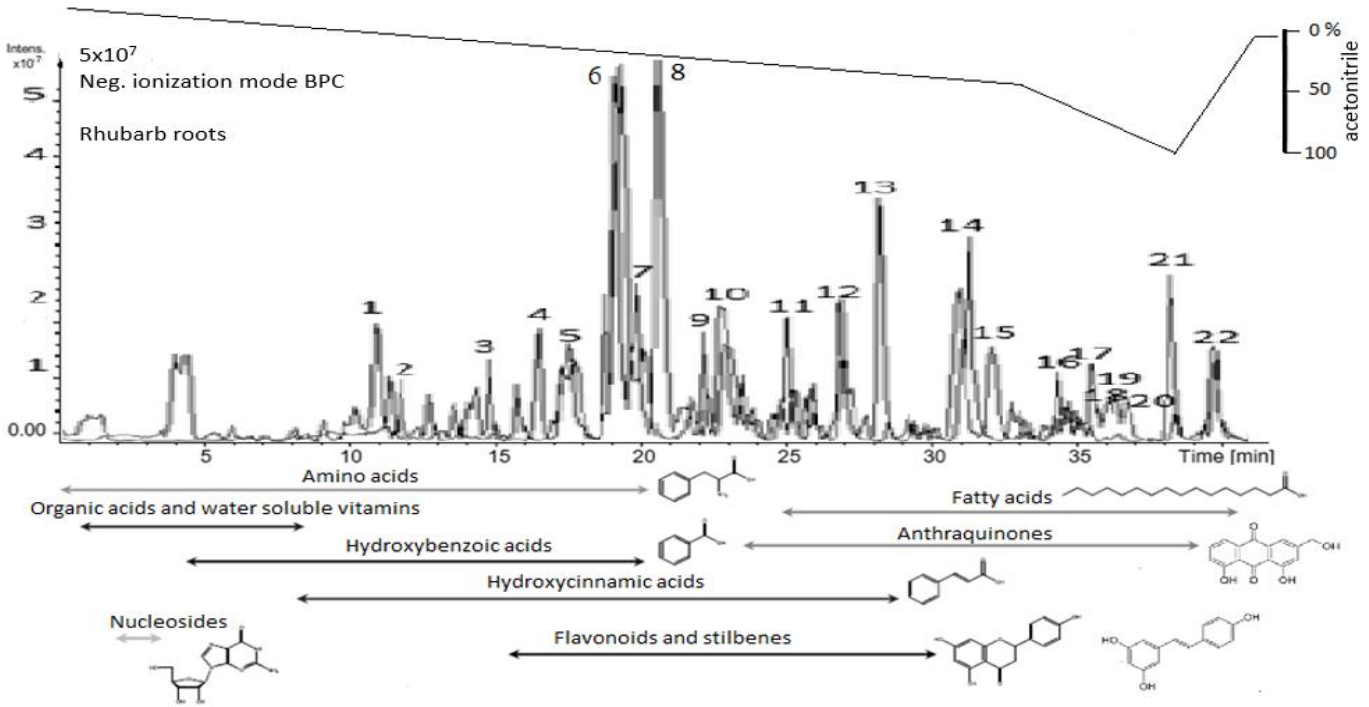


Figure 4. The approximate elution order of different compound classes in an example of rhubarb root extracts, using water (+formic acid) and acetonitrile binary gradient, the peak numbers refer to Fig. 7 and Table 3. The figure is adopted from Gómez-Romero *et al.* (2010)

2.5.2.5. High performance liquid chromatography method

The high performance liquid chromatography method enables analysis of compounds that are soluble in water-based liquids. For the chromatographic separation of polyphenolic compounds, usually reversed phase C_{18} columns and the binary mobile phase gradient system, composed of a polar aqueous phase and a less polar water-miscible phases, are used, gradually reducing the environment polarity. The gradient steps can vary, depending on the elution and separation properties of the compounds in the particular sample matrix (Fig. 4). The glycosides elute usually ahead of respective aglycones.

The quantification of polyphenolic compounds can be performed with a UV-Vis detector, coupled with an HPLC system, usually at 280 nm for total polyphenols, 350 nm for flavonols, 430 nm for anthraquinones, 500−520 nm for anthocyanins (Kapp *et al.*, 2013; Aleixandre-Tudo *et al.*, 2017). One or several standard compounds have to be used as reference, taking into account the retention times of the compounds.

In order to identify compounds, present in certain plant extracts, some preliminary knowledge is needed about the plant of interest and suitable standard compounds purchased. With a very good and steady chromatographic separation, it is possible to match the retention time of the standard compound with the compound in the sample even when using only the UV-Vis detector. For more accurate qualitative analysis in complex matrices such as food plants, MS-detectors should be used but the standard compounds still have to be used simultaneously, as the retention times or ionic weights of the compounds may be identical for several different compounds (de Rijke *et al.*, 2003). The HPLC system can be coupled to different types of mass detectors: quadrupole, triple quadrupole, ion trap or time of flight detectors. The first two types are more suitable for quantification of already known compounds and the latter two for identification and quantification of unknown compounds (de Rijke *et al.*, 2003).

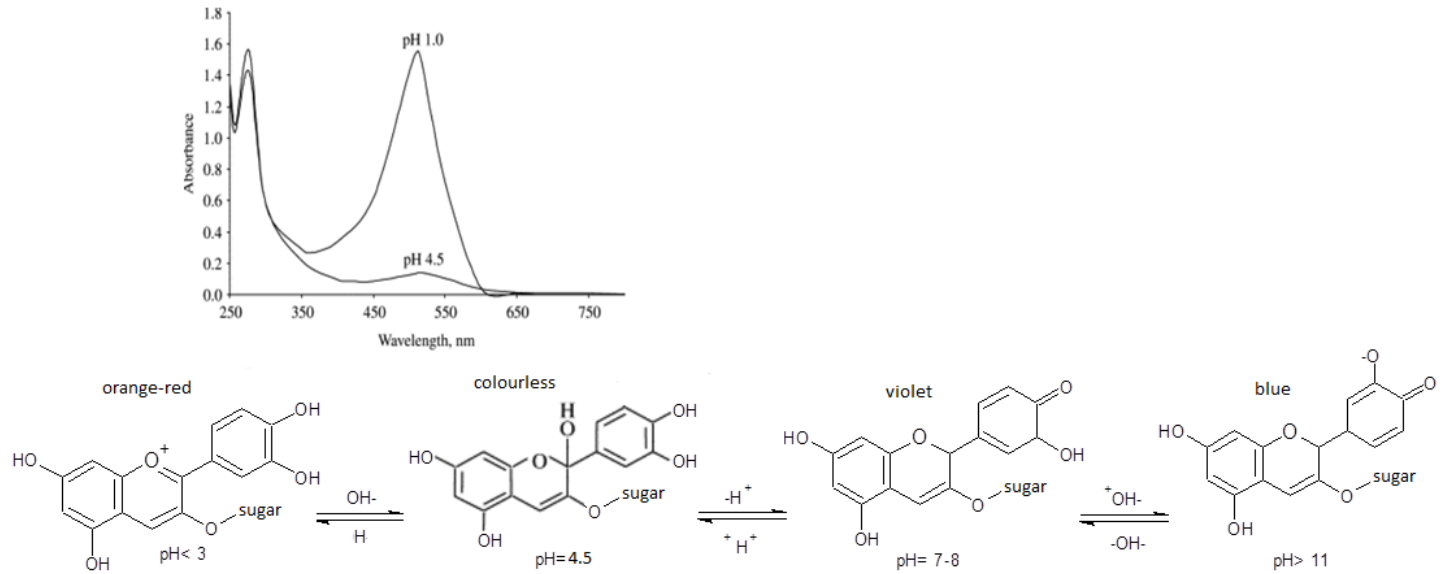


Figure 5. The UV-Vis absorption spectrum of anthocyanins, depending on the pH of the solution. The figure is adopted from Vankar and Srivastava (2010)

2.6. Antibacterial effect of polyphenols

2.6.1. Antibacterial mechanisms of polyphenols

Gram-negative (Gram−) bacteria have outer and inner membranes with a thin peptidoglycan layer in between (Fig. 6) which does not bind the staining colour crystal violet with Gram staining method. Gram-positive (Gram+) bacteria have only one cytoplasmic membrane, covered with a thick peptidoglycan layer (Fig. 6), which binds crystal violet colour and stains (Taguri *et al.*, 2006; Wilhelm *et al.*, 2015). These properties enable the first separation of the bacteria into two groups when identifying species, but also give some information about the bacterial cells´ possible permeability to other compounds, not just binding staining colour (Wilhelm *et al.*, 2015). According to Goni *et al.* (2009), Gram-negative bacteria are generally less susceptible to different antibacterial agents due to their outer lipopolysaccharide membrane (Fig. 6), which restricts diffusion of hydrophilic compounds into the bacterial cell. However, Taguri *et al.* (2006) found that the results of Gram staining do not correlate with the antibacterial (AB) susceptibility, it depended mostly on the particular bacterial species.

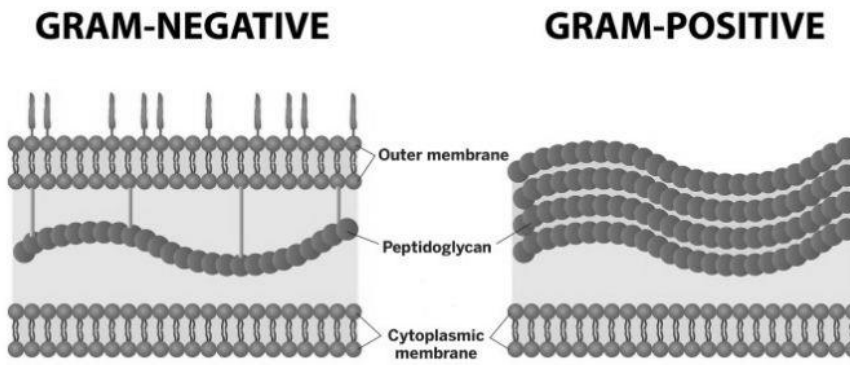


Figure 6. Gram− and Gram+ bacterial cell membrane differences. Figure by Shutterstock, 2021 (https://teachmephysiology.com/immune-system/infections/pathogens/)

2.6.2. Methods to assess antibacterial properties

2.6.2.1. Agar disk-diffusion method

The standard agar disk-diffusion method is available from the Clinical and Laboratory Standards Institute (CLSI, 2012), USA. In this method, a standard concentration of bacterial solution is spread in Petri dishes on the surface of an agar medium. Paper plates, impregnated with a certain amount of antibacterial compound, are then placed on the agar surface. After that, Petri dishes are incubated under conditions, appropriate to the bacterial species. During incubation, the antibacterial compound diffuses from the paper disks into the medium and inhibits bacterial growth around the disks. The potency of an antibacterial compound is assessed by measuring the radii or diameters of the resulting inhibitory zones (Balouiri *et al.*, 2016).

2.6.2.2. Agar well-diffusion method

In the agar well-diffusion method, the entire surface of the agar medium is coated with the test bacterial solution or the liquid agar medium broth is inoculated with the bacterial suspension before the agar solidifies. Sterile wells are then made in the medium to which 20–100 µl of the test antibacterial solutions are added. The agar plates are then incubated at a temperature appropriate for the bacterial species. The antibacterial compounds diffuse from the well into the medium and inhibit the germination or growth of the test bacterial species. The strength of the antibacterial effect is assessed by measuring the inhibitory zones' diameters or radii around the well (Balouiri *et al.*, 2016).

2.6.2.3. Minimal inhibitory concentration estimation method

Minimal inhibitory concentration (MIC value) is defined as the lowest concentration of an AB agent that inhibits the visible growth of the microorganism tested. It is usually expressed in µg/ml or mg/l. The MIC value is estimated by using different dilutions of the AB agent in the agar-well diffusion or microplate method, for example (Balouiri *et al.*, 2016).

2.6.2.4. Antibacterial properties of plant materials in the food matrices

AB properties of the plant material(s) in the food matrices can be estimated, for example, by enumeration of total or specific microbial counts in the products, during a defined time-period (Koskar *et al.*, 2019) and/or by a challenge test, where the growth potential of the pathogenic bacteria is estimated (Manou *et al.*, 1998).

2.7. Antioxidative effect and mechanisms of polyphenols

Polyphenolic compounds may act as antioxidants through several biochemical mechanisms. Polyphenols contain one or more benzene rings and two or more hydroxyl groups attached to the cycles (Fig. 2). Conjugated double bonds delocalize the electrons over a substantial part of the molecule. In this way, free radicals can be absorbed, stabilized and the radical chain reaction terminated. The more electron donating hydroxyl groups and conjugated double bonds in the molecule, the stronger antioxidant the molecule is (Huang *et al.*, 2005). Since in the glycosides, one or more hydroxyl groups are substituted with glucose or with some other saccharidic group, the aglycones are stronger antioxidants than glycosides (Csepregi *et al.*, 2016). In food matrices, both aglycones and glycosides can behave as antioxidative molecules but with different strength (Villano *et al.*, 2007; Csepregi *et al.*, 2016).

The second mechanism of antioxidativity is the molecule's ability to chelate pro-oxidant transition metal ions like ferrous (Fe^{2+}) or cuprous (Cu^{+}) ions. The structure of some polyphenols *e.g.* chalcones, allows them to chelate these ions and thereby reduce their potential as oxidative agents (Huang *et al.*, 2005). Potent chelating polyphenols must also have vicinal OH-groups in their molecule (Huang *et al.*, 2005).

Some polyphenol compounds may inhibit the oxidative enzymes like cyclooxygenases or lipoxidases and thus interrupt the oxidation reactions in the organism or in food systems (Eriksson, 1982; Actis-Goretta *et al.*, 2006; Altunkaya and Gökmen, 2008).

2.7.1. Methods for estimation of antioxidative capacity

The total antioxidative capacity (TAC) of a plant extract can be determined using a variety of different methods: the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, the ferric reducing antioxidant potential (FRAP) assay, the trolox equivalent antioxidant capacity (TEAC) where 2,21-azinobis(3-ethylbenzothiazoline)-6-sulfonate (ABTS) cation radical scavenging is measured, and by the Folin-Ciocalteu reagent reactivity (FCR). The latter is more commonly used for total polyphenol content estimation, but as this method actually measures overall reducing properties of the extract then Huang *et al.* (2005) have suggested using it for estimation of total antioxidativity.
In acidic conditions, the reducing capacity of antioxidative compounds may be suppressed due to protonation, whereas in alkaline conditions, proton dissociation of phenolic compounds would enhance a sample's reducing capacity (Huang *et al.*, 2005).

Csepregi *et al.* (2016) have correlated results of TEAC, FRAP, FCR and DPPH assays for 37 different phenolic compounds pairwise and found the correlation between FRAP and DPPH methods to be strong (R^2 = 0.81), the correlation between FCR and DPPH (R^2 = 0.45), between TEAC and DPPH (R^2 = 0.43) and between TEAC and FRAP (R^2 = 0.43) to be moderate. Correlations between TEAC and FCR and between FRAP and FCR were R^2 = 0.3 and R^2 = 0.4, respectively. All the correlations were statistically significant ($p < 0.001$). But, based on the results of Burri *et al.* (2017), strong positive correlations (Pearson) between the three methods measuring different antioxidant properties of plant extracts were found: between FCR and FRAP, R^2 = 0.958; between FCR-ABTS, R^2 = 0.980; and between FRAP and ABTS, R^2 = 0.957 ($p < 0.001$ in all occasions). The latter results suggest that, in the case of biological extracts, the correlations between different *in vitro* antioxidativity (AO) measurement methods can differ from correlations for pure compounds.

The results of radical scavenging assays are often expressed as half of the maximal effective concentration (EC_{50}), which is inversely related to the FRS capability of a compound. EC_{50} expresses the amount of antioxidant needed to decrease the radical concentration in the system by 50%. The lower the EC_{50}, the higher is the FRS capability of a compound (Villano *et al.*, 2007).

2.7.2. Estimation of antioxidative properties of a plant material in the food matrix

AO in the food matrix can be estimated by using the thiobarbituric acid reactive substances (TBARS) assay (Papastergiadis *et al.*, 2012), which measures the concentration of malondialdehyde as a principal secondary oxidation product of polyunsaturated fatty acids. The lipid oxidation primary products (Püssa *et al.*, 2009) or the FRS properties of different polyphenols in food samples can be simultaneously detected and quantified by high performance liquid chromatography, coupled with a mass selective detector with an additional product ion analyzer (HPLC-MS/MS) (Bandonienė and Murkovic, 2002).

2.8. Garden rhubarb (*Rheum rhaponticum* L.)

2.8.1. Taxonomy of garden rhubarb

Rheum rhaponticum L. is classified botanically as:
Kingdom: *Plantae*
Division: *Angiosperms*
Class: *Eudicots*
Order: *Caryophyllales*
Family: *Polygonaceae*
Genus: *Rheum*
Species: *Rheum rhaponticum* L.
(Flora of North America, 2005)

2.8.2. Practical use of garden rhubarb

Rhubarb petioles are used in making pies, marmalades, juices and marinades. Sometimes the plant is used in jams to acidify other fruits and vegetables that are less sour, *e.g.* strawberries, apples or pumpkin (Flora of North America, 2005).

Infusions or fermented infusions of rhubarb leaves are used in organic plant protection and as leaf fertilizer (Reuveni and Reuveni, 1998; Wang *et al.*, 2007). The leaves are not edible for humans due to their high content of toxic oxalic acid (Kalliala and Kauste, 1964).

Rhubarb root extract has been used for treatment of perimenopausal symptoms and as a mild laxative (Hasper *et al.*, 2009). The nutritional composition of rhubarb petioles, according to the food composition database NutriData (2019), is shown in Table 1.

2.8.3. Polyphenols of garden rhubarb

The polyphenolic composition and the quantity of different compounds of different garden rhubarb cultivars may be somewhat different (Rumpunen and Henriksen, 1999). Nevertheless, there are some major groups of compounds that are generally present in roots and petioles of all cultivars like *trans*-stilbenes (resveratrol, piceatannol, rhapontigenin and their *O*-glucosides) and anthraquinones (emodin, chrysophanol, and their glucosides) and torachysone (Kolodziejczyk-Czepas *et al.*, 2020). In the petioles, the major flavonols are catechin, epicatechin, epigallocatechin gallate or gallocatechin gallate, myricetin-*O*-rhamnoside, quercetin-*O*-rutinoside (rutin), quercetin-*O*-glucuronide, quercetin-*O*-glucoside, quercetin-*O*-rhamnoside and quercetin (Komatsu *et al.*, 2006). If the anthocyanins are present in the petioles (depending on the cultivar), then these are cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside (Takeoka *et al.*, 2013). Takeoka *et al.* (2013) have measured the total content of anthocyanins in rhubarb petioles 19−341 mg in 100 g dry weight (DW).

2.8.4. Antibacterial properties of garden rhubarb

Li *et al.* (2016) have shown that hydrophobic hydroxyanthraquinone − emodin may destroy bacterial cell membrane integrity by influencing the conformation of membrane proteins and thus increase membrane permeability. In rhubarb roots, hydroxyanthraquinones are quantitatively the major active components, having many biological and pharmacological properties including AB properties (Iizuka *et al.*, 2004; Wang *et al.*, 2010). In the study of Lu *et al.* (2011), the minimum inhibitory concentration (MIC) of crude extracts of rhubarb root was positively related to hydroxyanthraquinones content, and it was found that rhubarb may have potential use as an AB agent for control of some pathogenic bacteria.

In the case of rhubarb petioles, as it may be concluded from the results of Colla *et al.* (2018), Daglia (2012) and Ma *et al.* (2018), the AB effect

could be connected besides flavanols *e.g.* epicatechin, epigallocatechin-gallate and *trans*-stilbenes, with relatively high nitrate ion content, that can be turned into antimicrobial nitrites. In addition, organic acids lower the pH of the media and therefore make the environment intolerable for some bacterial species (Hmelak Gorenjak and Cencič, 2013; Colla *et al.*, 2018).

2.8.5. Free radical scavenging properties of garden rhubarb

In rhubarb petioles, the AO properties can be related to the content of ascorbic acid, flavonoids and *trans*-stilbenes (Miura *et al.*, 2000; Yen *et al.*, 2002; Kalisz *et al.*, 2020).

2.9. Blackcurrant (*Ribes nigrum* L.)

2.9.1. Taxonomy of blackcurrant

Blackcurrant is classified botanically as:
Kingdom – *Plantae*
Division – *Magnoliophyta, Tracheophyta*
Subdivision – *Spermatophytina*
Class – *Magnoliopsida*
Superorder - *Saxifraganae*
Order – *Saxifragales*
Family – *Grossulariaceae*
Genus – *Ribes*
Sub-genus – *Coreosma*
Species – *Ribes nigrum* L.
(EU-NOMEN, 2021; Vagiri, 2014)

2.9.2. Practical use of blackcurrant leaves and berries

The leaves of blackcurrant have been used in folk medicine for their diaphoretic and diuretic properties as well as their ability to relieve rheumatic pain (European Scientific Cooperative on Phytotherapy (ESCOP), 2003). Their pharmacological effects, other than their anti-inflammatory effect have not been scientifically proven (Declume, 1989; Garbacki *et al.*, 2005). The anti-inflammatory effect may be due to the polyphenolic compounds in the leaves (Grayer and Kokubun, 2001). Surveys have indicated that some of the polyphenols may be exposed on

the cuticle of leaves, as antimicrobial agents against microbial attack (Witzell *et al.*, 2003). Blackcurrant leaves are widely in use as a tea material (Bisset and Wichtl, 1994) and for the "Louhisaari" alcoholic drink, prepared as a sweetened vodka infusion from young leaves (Vagiri, 2014). Blackcurrant berries can be used to make jams, sweets and juice or they can be consumed fresh as dessert berries. The nutritional composition of blackcurrant berries, according to the food composition database NutriData (2019), is shown in Table 1.

2.9.3. Polyphenols of blackcurrant

In blackcurrant berries, leaves and buds, the following polyphenolic compounds have been found: chlorogenic acid, *neo*-chlorogenic acid, catechin, epicatechin, epigallocatechin, anthocyanins: delphinidin-3-*O*-glucoside, delphinidin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside (Vagiri *et al.*, 2012; Mattila *et al.*, 2016). Tian *et al.* (2019) found additionally pelargonidin 3-*O*-glucoside, pelargonidin 3-*O*-rutinoside, peonidin 3-*O*-glucoside, peonidin 3-*O*-rutinoside, malvidin 3-*O*-glucoside, malvidin 3-*O*-rutinoside, delphinidin 3-*O*-(6"-coumaroyl)-glucoside and cyanidin 3-*O*-(6"-coumaroyl)-glucoside. Nour *et al.* (2013) found 200 to 300 mg/100 g FW of anthocyanins in ripe blackcurrant berries. Vagiri *et al.* (2012), Mattila *et al.* (2016) and Tian *et al.* (2019) found myricetin malonylglucoside (two isomers), quercetin-3-*O*-rutinoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, quercetin-3-6-malonyl-glucoside, kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-glucoside, isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside, kaempferol-malonylglucoside (two isomers), and quercetin in blackcurrant. In bud extracts, 3-*O*-caffeoylquinic acid (*neo*-chlorogenic acid), chlorogenic acid and additionally, *p*-coumaroylquinic acid, 4-*O*-caffeoylquinic acid (crypto-chlorogenic acid), caffeic acid, *p*-coumaroylquinic acid, *p*-coumaroyl quinic acid 2, *p*-coumaric acid, ferulic acid, caffeic acid ethylester, cinnamic acid derivative, gallic acid and taxifolins 1–4 have been detected (Ieri *et al.*, 2015). Blackcurrant berries contain, besides polyphenols organic acids, citric acid, malic acid, tartaric acid, fumaric acid and shikimic acid, on average: 1.26; 0.73; 0.14; 0.95 and 2.65 mmol/100 g FW, respectively (Mikulic-Petkovsek *et al.*, 2012) and ascorbic acid 148–310 mg/100 g fresh weight (FW) (Vagiri *et al.*, 2013). These compounds may also contribute to the *in vitro* properties of the berries. In blackcurrant leaves or buds, additionally to the

different polyphenolics, volatile compounds give input to the *in vitro* properties (Dvaranauskaite *et al.*, 2008).

2.9.4. Antibacterial properties of blackcurrant

Nowak *et al.* (2016) used blackcurrant leaves in meat products for antimicrobial effect; however, the leaves of the sour cherry proved to be more effective in this study. Stević *et al.* (2010) used the essential oil fraction of blackcurrant leaves, which yielded 0.12% of the leaf mass, for the AB effect. They found that *E. coli* was almost two times more sensitive to blackcurrant leaf essential oil compared to the control streptomycin (1 mg/ml; 16 µl), but the MIC value for *L. monocytogenes* was 10 µl higher compared to the control streptomycin (1 mg/ml; 16 µl).

2.9.5. Free radical scavenging properties of blackcurrant

Jakobek *et al.* (2007) compared ten different species: fruits of blackcurrant (*Ribes nigrum* L.*)*, red currant (*Ribes rubrum* L.), red raspberry (*Rubus idaeus* L.), blackberry (*Rubus fruticosus* L.), sour cherry (*Prunus cerasus* L.), sweet cherry (*Prunus avium* L.), strawberry (*Fragaria × ananassa* (Duchesne ex Weston) Duchesne ex Rozier), chokeberry (*Aronia melanocarpa* (Michx.) Elliott), elderberry (*Sambucus nigra* L.) and bilberry (*Vaccinium myrtillus* L.*)* in terms of FRS properties. They found that blackcurrant berries were third best in ABTS and DPPH radical scavenging properties after chokeberry and bilberry. All of these three berry species were selected for the different experiments of the present thesis.

2.10. The other plants in comparison

2.10.1. Blue honeysuckle berries, *Lonicera caerulea* L. *var. edulis* Turcz. *ex* Herder

Blue honeysuckle or haskap berry has been cultivated in Estonia since 1980. With the analyses of 20 different blue honeysuckle cultivars (Arus *et al.*, 2018), it was found that their berries contained 1.5–7% of titratable acids and the dominant organic acid was citric acid – up to 47% of total organic acids, followed by 5% oxalic acid, and other acids. The total amount of dry matter in the ripened berries is 14.3% on the average. Blue honeysuckle berries contain sugars 6.9% on the average, 80% of

which are glucose and fructose. The pH of the berries is between 2 to 3. The dry matter/titratable acid ratio of the berries is 7.7 on the average, which results in relatively sour taste of the berries. The vitamin C content has been measured 15–88 mg in 100 g FW that is 44 mg per 100 g on the average (Arus *et al.*, 2018).

2.10.1.1. Polyphenols of blue honeysuckle berries

In parallel studies to the present doctoral study, the total anthocyanin content of blue honeysuckle berries were compared with several other anthocyanin containing species and it was found that blue honeysuckle berries contain anthocyanins at 601 mg/100 g FW (Raudsepp *et al.*, 2017). This result placed blue honeysuckle on the second position after chokeberry, which contained the highest amount of anthocyanins of all studied berries in the abovementioned study. However, in the recent honeysuckle study of Česonienė *et al.* (2021), only 343 mg/100 g FW of anthocyanins were measured on the average for 11 cultivars. The following description of the general polyphenolic prophile of *L. caerulea* is largely based on a comprehensive review of several blue honeysuckle research papers by Rupasinghe *et al.* (2018), Chaovanalikit *et al.* (2004) and additionally on the research of Šenica *et al.* (2018).

Phenolic acids: caffeic acid, chlorogenic acid (3-caffeoylquinic acid), neochlorogenic acid, 3,5-dicaffeoylquinic acid, protocatechuic acid, *m*-coumaric acid, salicylic acid.

Anthocyanins: cyanidin-3-*O*-glucoside (C3G, chrysanthemin), cyanidin-3,5-diglucoside, cyanidin-3-*O*-rutinoside, pelargonidin-3-*O*-glucoside, peonidin-3-*O*-glucoside.

Other flavonoids: quercetin, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-rutinoside (rutin), quercetin-3-*O*-rhamnoside (quercitrin), luteolin 7-*O*-α-glucoside, catechin, epicatechin, procyanidins.

Other polyphenols: Catalposide, resveratrol (Jurikova *et al.*, 2012), using electrochemical, not mass- spectrometric detection.

Šenica *et al.* (2018) identified the following polyphenolic compounds in blue honeysuckle berries: isorhamnetin acetylhexoside; isorhamnetin

acetyl rhamnosyl hexoside; isorhamnetin hexosyl pentoside; isorhamnetin-3-rutinoside; kaempferol acetylhexoside; kaempferol hexosyl pentoside; kaempferol 3-*O*-galactoside; kaempferol-3-*O*-glucoside; quercetin-3-*O*-acetyl hexoside; quercetin-3-*O*-arabino-furanoside; quercetin-3-*O*-galactoside; quercetin-3-*O*-glucoside quercetin-glycoside; quercetin-3-*O*-hexoside; quercetin-3-*O*-hexosyl-pentoside; quercetin-3-*O*-rutinoside; quercetin-3-*O*-vicianoside; quercetin-3-*O*-xyloside; naringenin hexoside; loganin-7-*O*-pentoside and also procyanidins.

2.10.2. Chokeberry berries, *Aronia melanocarpa* (Michx.) Elliott

Chokeberry is usually grown in Estonia as seedlings of the hybrid species *Aronia melanocarpa* (Michx.) Elliott, not as registered cultivars (personal communication with Mrs Hedi Kaldmäe; Eestikeelsete taimenimede andmebaas, *Aronia*, 2021), therefore the chemical composition and properties of the berries may vary widely. The average nutritional composition of chokeberry berries, according to the food composition database NutriData (2019), is shown in Table 1.

2.10.2.1. Polyphenols of the chokeberry berries

Chokeberry berries contain more than 1,100 mg/100 g FW (Raudsepp *et al.*, 2017) of anthocyanins, all glycosides of cyanidin: cyanidin 3-*O*-glucoside, cyanidin 3-*O*-galactoside, cyanidin 3-*O*-arabinoside, and cyanidin 3-*O*-xyloside (Bräunlich *et al.*, 2013). Also two phenolic acids: chlorogenic acid and neochlorogenic acid (Oszmiański and Wojdylo, 2005; Wangensteen *et al.*, 2014), and different quercetin glycosides: quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, quercetin 3-*O*-rutinoside, quercetin 3-*O*-robinobioside and quercetin 3-*O*-vicianoside (Slimestad *et al.*, 2005; Girones-Vilaplana *et al.*, 2012; Lee *et al.*, 2014) are found in chokeberry berries.

2.10.3. Bilberry berries, *Vaccinium myrtillus* L.

Bilberry is native mostly to Europe with only a few small natural growing areas in North America (Vander Kloet, 1988). It is often wrongly called blueberry, which is the English name for the species *Vaccinium angustifolium* Ait. and *Vaccinium corymbosum* L., both of which are native to

North America (Vander Kloet, 1988), although also widely cultivated in Europe. Bilberry berries contain 86% water. Their dry matter comprises 10.2 g absorbable carbohydrates, 3.3 g fibre, 1.4 g organic acids, 10 mg vitamin C, 310.5 μg carotenoids and 1.9 mg vitamin E in 100 g FW (FINELI, 2021). The nutritional composition of bilberry berries, according to the food composition database NutriData (2019), is shown in Table 1.

2.10.3.1. Polyphenols of bilberry berries

Bilberry berries contain anthocyanins, depending on their maturity and location, with more than 500 mg (Pepkolaj *et al.*, 2017; Raudsepp *et al.*, 2017) in 100 g FW. Anthocyanins occur in bilberries as a mixture of the five most known anthocyanidins and their glycosides − anthocyanins: glycosides of cyanidin and delphinidin are quantitatively the major compounds; followed by glycosides of malvidin, petunidin, and peonidin; together they constitute 95% of total polyphenols, found in bilberry berries (Scalzo *et al.*, 2008; Tian *et al.*, 2017). According to the European Pharmacopoeia (2019), bilberries contain the following anthocyanins and anthocyanidins: delphinidin-3-*O*-galactoside, delphinidin-3-*O*-glucoside, cyanidin-3-*O*-galactoside, delphinidin-3-*O*-arabinoside, cyanidin-3-*O*-glucoside, petunidin-3-*O*-galactoside, cyanidin-3-*O*-arabinoside, petunidin-3-*O*-glucoside, delphinidin, peonidin-3-*O*-galactoside, petunidin-3-*O*-arabinoside, peonidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, peonidin-3-*O*-arabinoside, malvidin-3-*O*-galactoside, cyanidin, malvidin-3-*O*-arabinoside, petunidin, peonidin and malvidin.

Additionally to anthocyanins, bilberries contain other polyphenols: quercetin derivatives, ellagic acid, ferulic acid and its glycosides, chlorogenic acid, caffeic acid, myricetin and its glycosides, syringic acid, vanillic acid, cinnamic acid and other compounds (Aaby *et al.*, 2013).

2.10.4. Tomato fruits, *Solanum lycopersicum* L.

Perveen *et al.* (2015) and Gómez-Romero *et al.* (2007) described the nutritional profile of tomatoes. Marconi *et al.* (2007) described the organic acid profile of tomato fruits; they found malic, pyruvic, lactic, acetic, citric, pyroglutamic, succinic, citramalic, fumaric and *t*-aconitic

acids. The nutritional composition of tomato fruits, according to the food composition database NutriData (2019), is shown in Table 1.

2.10.4.1. Polyphenols of the tomato fruits

Anton *et al.* (2017) analysed the polyphenolic composition of tomatoes. They detected the following compounds: caffeic acid hexosides, 3-(2-hydroxyphenyl) propanoic acid hexoside, homovanillic acid hexoside, *p*-coumaric acid hexoside, neo-, crypto and chlorogenic acid, ferulic acid hexoside, coumaroylquinic acid, rutin hexoside, apigenin acetylhexoside, quercetin dihexoside, eriodictyol hexoside, rutin pentoside, quercetin rutinoside (rutin), kaempferol rutinoside pentoside, phloretin dihexoside, naringenin hexoside, dicaffeoylquinic acids, kaempferol rutinoside, naringin, caffeic acid derivative, dihydrocaffeic acid dihexoside, eriodictyol, naringenin, tricaffeoylquinic acid and naringenin chalcone. Results of Anton *et al.* (2017) concur with those of Gómez-Romero *et al.* (2010).

2.10.5. Sea buckthorn berries, *Hippophae rhamnoides* L.

Depending on the cultivation location, whether the berries are fresh or frozen prior to analysis and on the speed of analysis, sea buckthorn berries may contain 49 to 360 mg/100 g FW of vitamin C (Jalakas *et al.*, 2003; FINELI, 2021). Berries also contain 3 mg of vitamin E and 158.6 μg of carotenoids in 100 g FW. All of these compounds, additionally to phenolic compounds, may also contribute to the FRS and other properties of these berries. The average nutritional composition of sea buckthorn berries, according to the food composition database NutriData (2019), is shown in Table 1.

2.10.5.1. Polyphenols of the sea buckthorn berries

Nadõrova (2007) identified the following polyphenolic compounds: rhamnetin and isorhamnetin glycosides, quercetin and its rutinoside and their glycosides, prodelphinidin, catechin and epicatechin, kaempferol, syringing, myricetin, and its glycosides and different procyanidins in sea buckthorn (SB) berries. Pop *et al.* (2013) found similar compounds in SB berries.

Table 1. Nutritional composition (in 100 g of FW) of the studied food plants, except of blue honeysuckle berries, available in the food composition database (NutriData, 2019)

Nutrient	GR petioles	BC berries	CB berries	BB berries	TOM fruits	SB berries	RDA
Macro-nutrients							
Energy, kJ	140	213	210	248	92	352	8,380
Energy, kcal	33.3	50.8	50.1	59	21.9	85	2,000
Carbohydrates, g	9.3	13.6	14.4	13.5	4.9	12.3	270
Lipids, g	0.1	0.4	0.1	0.8	0.3	5	66
Fibers, g	3.8	5.8	5.6	3.3	1.4	6	30
Proteins, g	0.7	1.1	0.7	1.1	0.6	0.7	73
Water, g	90.5	77.9	84.4	86	94.7	82.6	1,500
Carbohydrates							
Absorbable carbohydrates, g	5.5	7.8	8.8	10.2	3.5	6.3	
Starch, g	0	0	-	1.8	0.1	0	
Sugars (∑), g	5.5	8.5	0.5	8.4	3.4	6.3	
Sacharose, g	5	0.6	0.5	0.1	0.1	0.1	
Glucose, g	0.2	3.5	-	3.7	1.3	3.7	
Fructose, g	0.3	4.4	-	4.6	2	2.5	
Lipids							
Fatty acids (∑), g	0	0.3	0	0.5	0.06	2.74	
Saturated fatty acids, g	0	0.1	0	0.1	0	0.8	max 22
MUFAs, g	0	0	0	0.1	0	1.6	33
PUFAs, g	0	0.2	0	0.3	0.06	0.34	16
Palmitic acid, g	0	0.1	0	0	0	-	
Linoleic acid, g	0	0.1	0	0.2	0.06	0.25	
Linolenic acid, g	0	0.1	0	0.1	0	0.09	
Cholesterol, mg	0	0	0	0	0	0	max 300
Minerals							
Sodium, mg	1.2	0.5	3	0.3	2.5	3.5	575–2,400
Potassium, mg	320	340	218	110	290	133	min 3,100
Calcium, mg	75	72	32	19	9	42	min 800
Magnesium, mg	9	24	16	9	11	30	min 320
Phosphorus, mg	17	58	72	20	30	8.6	max 3,000
Iron, mg	0.1	1.2	0.93	0.6	0.3	0.4	min 15
Zinc, mg	0.2	0.3	0.3	0.2	0.2	0	min 9
Copper, mg	0.06	0.1	-	0.11	0.06	-	
Manganese, mg	0.17	0.3	-	4.2	0.09	-	
Iodine, μg	1	1	2	1	0.2	0	min 150
Selenium, μg	0.2	0	-	0.25	0	0.1	min 50
Chromium, μg	0.6	0.8	-	0.3	1.5	-	
Nickel, μg	7.2	9.6	-	7	3.2	-	

Nutrient	GR petioles	BC berries	CB berries	BB berries	TOM fruits	SB berries	RDA
Vitamins							
Vitamin A, RE	9.79	8.2	93.8	3.9	43	2.6	min 700
Retinol, μg	0	0	0	0	0	0	
β- carotene equivalents	118	98	1,130	47	518	31	
Vitamin D, μg	0	0	0	0	0	0	min 10
Vitamin D3, μg	0	0	0	0	0	0	
Vitamin E, αTE	0.35	2.2	1.5	1.9	0.74	3	min 8
Vitamin K, μg	29	30	22	12	5	11	
Vitamin B1, mg	0.03	0.05	0.02	0.04	0.06	0.18	
Vitamin B2, mg	0.03	0.07	0.02	0.07	0.04	0.07	min 1.3
Niacin equivalents B3, (NE) (sum)	0.48	0.5	0.4	0.4	0.85	0.4	min 15
Niacin, mg	0.3	0.3	0.3	0.3	0.7	0.3	
Niacin equivalents from tryptophan, mg	0.18	0.2	0.1	0.1	0.15	0.1	
Pantothenic acid B5, mg	0.09	0.4	-	0.16	0.19	0.15	
Vitamin B6, mg	0.04	0.17	0.03	0.07	0.1	0.13	min 1.5
Biotin B7, μg	-	2.4	-	1.1	1.4	3.3	
Folates B9, μg	7	7.7	7	12	17	10	min 300
Vitamin B12, μg	0	0	0	0	0	0	min 3
Vitamiin C, mg	5	150	28	15	14.1	165	min 100

BB − bilberry, BC − blackcurrant, CB − chokeberry, GR − garden rhubarb, TOM − tomato, MUFAs − monounsaturated fatty acids, NE – niacin equivalents, PUFAs − polyunsaturated fatty acids, RE – retinol equivalents, RDA − Recommended daily allowance of a nutrient, SB − sea buckthorn, TE − tocopherol equivalents

3. AIMS OF THE STUDY

The main aim of the present study was to find, among some pre-selected food plant materials, the one(s) that has/have both high antibacterial (AB) and free radical scavenging (FRS) properties.

The specific aims of this study were as follows:

1. to determine the polyphenolic composition of garden rhubarb roots and petioles (**I**, **IV**).

2. to determine the polyphenolic composition of blackcurrant berries and leaves (**II**, **IV**).

3. to compare different plant materials in relation to their AB and FRS properties:

    a) to compare the petioles and roots of garden rhubarb and the fruits of blue honeysuckle, tomato, bilberry, sea buckthorn, and blackcurrant (**III**).

    b) to compare the leaves and berries of blackcurrant; berries of chokeberry and blue honeysuckle; petioles and roots of garden rhubarb (**IV**).

4. MATERIALS AND METHODS

4.1. Preparation of the rhubarb roots and petioles for polyphenol analyses

For paper **I**, samples (batches of 50 g each) of roots and petioles of rhubarb (*R. rhaponticum* L.) were collected near Kuusalu in Northern Estonia. Voucher specimens (no. 715) of the rhubarb roots and petioles are deposited at the Institute of Pharmacy, University of Tartu, Nooruse 1, Tartu 50411, Estonia. After cutting into pieces with maximum dimensions 1×5 cm, the plant material was air-dried. The dried samples with a residual moisture content of 18±2% were powdered in a mortar, the powder sieved through a 1 mm sieve and two parallel sub-samples comprising 1 g each were macerated with a 10-fold excess (w/v) of methanol (> 99.9%) for 72 h at room temperature with periodic shaking. This was followed by centrifugation in a cooling centrifuge Eppendorf 5810R (Eppendorf AG), equipped with a swinging bucket rotor, for 15 min at 978 *g*, the supernatants were kept at −20°C. After removal of the supernatant and short-term rinsing with 2 ml of water, the sediment was re-extracted with another 10 ml of methanol. In paper **III** the rhubarb specimen and in paper **IV**, seedlings (101 and 303) were used together with cultivars ´Victoria´ and ´Ogres´ from the resources of Polli Horticultural Reasearch Centre. The selection was made from among 16 seedlings or cultivars, based on their total polyphenol and hydroxyanthraquinone content. For paper **IV** all the dried plant materials were powdered with a blender (Stollar/Kinetix®Control) to a particle size diameter of ≤3 mm; the necessary fraction was obtained with an analytical sieve shaker AS300 control (Retsch GmbH). For the infusions, 1 g of each powder in duplicate was mixed with 20 ml of 20% and 96% aqueous ethanol. The mixtures were rotated on a Multi RS-60 Multirotator (Biosan) at 40 rpm for 24 h at room temperature, followed by centrifugation at 2,594 *g* for 10 min on a Sigma 4-16KS (Sigma Laborzentrifugen GmbH) centrifuge. The supernatants were collected and further diluted by two, four and eight times for estimation of AB and FRS properties, and for quantitation of total polyphenol content (TPC).

4.2. Preparation of the blackcurrant berries and leaves for polyphenol analyses

Blackcurrant (*Ribes nigrum* L.) was represented in the study by several cultivars. In the compositional study of leaves of blackcurrant in paper **II**, the cultivar ´Karri´ was used. Cultivar ´Karri´ (´Mulgi must´ x ´Kantata´; 1990) was bred by A. Libek in Estonia and registered in 2003 (Libek *et al.*, 2008; Libek *et al.*, 2017). In paper **IV**, the leaves of cultivar ´Pamyati Vavilova´ were used as these leaves were also collected for industrial use, due to the large number of bushes of this cultivar compared to the other cultivars. Fully developed leaves for the qualitative analysis of polyphenols were collected randomly from 6 different bushes of the same cultivar including equal proportions of leaves from different sides and inner and outer parts of the bushes.

For paper **II**, 500 g of berry samples per cultivar were collected in the middle of the harvesting season from the cultivar evaluation test plots established in 2000. Four prospective selections (10B, 2-96-51, 1-96-16, 4-96-1) and 4 new cultivars (´Karri´, ´Almo´, ´Ats´, ´Elo´) from the Estonian breeding programme and 7 introduced cultivars (´Öjebyn´, ´Zagadka´, ´Ben Sarek´, ´Intercontinental´, ´Pamyati Vavilova´, ´Titania´ and ´Pilenai´) were analysed in three consecutive years 2005, 2006 and 2007. For paper **IV** ´Ben Alder´ berries (among 37 cultivars) were selected for the tests according to their highest content of anthocyanins. The cultivar ´Ben Alder´ was bred in the Scottish Crop Research Institute, by crossing the cultivars ´Ben More´ and ´Ben Lomond´ (Kikas *et al.*, 2008). For the anthocyanidin analysis, 200 g of fresh frozen berries were homogenized with a Heidolph DIAX 900 homogenizer, 2 g of the sample was weighed with an analytical standard scale (OHAUS) into a 50 ml centrifuge tube, two parallel samples of each. In the present work (**II**, **III**, **IV**), mainly food grade extracting solvents (water and ethyl alcohol at different concentrations) were used, as the following studies were meant to be conducted with the same plant extracts in the food matrices. The samples were extracted for 30 min at room temperature with 20 ml ethanol/water/HCl (70:30:1), after which the samples were shaken on a minishaker IKA MS 1 (IKA-WORKS) for 30 s twice after 5 min. The tubes were then centrifuged with an Eppendorf centrifuge 5810 R at 3,220 *g* at 20ºC for 10 min, the supernatant was removed and the samples were extracted once more with 20 ml ethanol/water/HCl (70:30:1) using the same procedure, and

for the third time with 5 ml ethanol/water/HCl (70:30:1). The supernatants were combined and 1 ml of the final solution was filtered through a Spartan 13 mm regenerated cellulose membrane filter with a pore size of 0.2 μm. For the acid hydrolysis of the samples, the final filtrate was taken to concentration 1 M of HCl, and heated in a Binder oven at 90ºC for 60 min (**II**). For the polyphenol analysis of the leaves 0.1 g of dried leaves, three parallel samples, were weighed into a 15 ml centrifuge tube, and 10 ml of 40% ethanol was added. The samples were extracted at room temperature for 24 h. The extracts were centrifuged at 3,220 *g* at 20ºC for 10 min. The supernatant was filtered through a Spartan 13 mm regenerated cellulose membrane filter with a pore size 0.2 μm (**II**).

4.3. Chromatographic conditions

The plant samples were analysed using liquid chromatography electrospray ionization tandem mass spectrometry (LC-DAD-ESI/MS^2) on an Agilent 1100 Series LC/MSD ion Trap-XCT (Agilent Technologies). The ion trap was connected to the HPLC instrument consisting of an autosampler, solvent membrane degasser, binary pump and column thermostat. The HPLC 2D ChemStation software with a ChemStation Spectral SW module was used both for process guidance and processing the results. The reversed-phase-HPLC analytical separation was performed on an Agilent Technologies Zorbax 300SB-C18 column (150×2.1 mm i.d.; particle size 5 μm) with a guard column filled with the same type of sorbent, in a stepwise gradient mode of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) at the flow rate of 0.3 ml/min at 35°C. Elution was started with a linear gradient of B from 10 to 30% with 30 min, then to 90% by 40 min and finished isocratically with 90% of B for 10 min. The analysis was conducted with negative ionisation mode (**I**, **II**, **III**, **IV**), except for anthocyanins (**II**, **IV**), where positive ionization mode and higher content (1%) of formic acid in water was used as solvent A. The UV-Vis photodiode-array detector recorded in the interval 200–600 nm and the eluate optical density (OD) was continuously monitored at wavelengths 280 nm (flavanols), 306 nm (*trans*-hydroxystilbenes), 370 nm (flavonols), 430 nm (hydroxyanthraquinones) and 510−520 nm (anthocyanins). The conditions of MS^2 detection were as follows: m/z interval 50–1,000; target mass, 400; number of fragmented ions, two; maximal accumulation time, 100 ms; compound stability, 100%; drying gas

(nitrogen from generator) flow rate, 10 l/min; gas temperature, 350°C; nebuliser pressure, 30 psi; collision gas helium pressure, 6×10^{-6} mbar.

The TPC and anthocyanin content (**IV**) of plant infusions were estimated by areas under UV-Vis chromatographic curves at 280 and 520 nm, respectively, using an ultra-high-performance liquid chromatographic–mass spectrometric Shimadzu Nexera X2 system (Shimadzu Scientific Instruments). For the estimation of TPC and anthocyanin content, chlorogenic acid (Aldrich) and cyanidin- 3-*O*-glucoside chloride (kuromanin chloride, Sigma) calibration curves were used, respectively. On some of the chromatogram figures, illustrating the results, the void time peaks are cut off, to show more clearly the peaks of the analytes. Therefore the retention times are also not shown on these figures.

4.4. Preparation of the plant materials for *in vitro* analyses

For the evaluation of FRS and AB properties (**III**), the freeze-dried fruits of blue honeysuckle, tomato, bilberry, sea buckthorn, and blackcurrant and thermally dried (at 45°C) petioles and roots of the Siberian rhubarb were treated as follows: plant materials were ground and further treated by two different methods:

A) the decoction method (Bisset and Wichtl, 1994), similarly to the home-made tea preparation: to the dry material of 500 mg aqueous phosphate buffer (pH = 7) was added in the ratio 1:10 (w/v) and the mixture was heated at 95°C for 10 min. The obtained infusion was cooled down and centrifuged at 3,220 *g* for 10 min on the Eppendorf 5810R cooling centrifuge. The obtained supernatant was centrifuged once more and diluted for the measurements up to the ratio 1:80 (w/v).

B) dry plant material was macerated in 10 fold excess (w/v) of 30% ethanol at room temperature for 24 h with periodic shaking (18 r/min) on a rotating shaker (Multi RS-60, BIOSAN). The obtained infusion was centrifuged at 3,220 *g* on the Eppendorf 5810R cooling centrifuge. The obtained supernatant was centrifuged once more and diluted for the measurements up to 1:80 (w/v). For HPLC analysis 1:20 (w/v) dilutions, for antioxidant efficiency 1:40 (w/v)

dilutions and for antimicrobial properties analyses 1:20, 1:40 and 1:80 (w/v) dilutions together.

The buffered water was used as one extracting solvent to minimize the pH influence (**III**). However, in paper **IV**, pH of the extracting solvent was not altered to compare the actual reducing properties of the 20% and 96% ethanolic extracts of studied plants.

All of the studied plants, collected for AB and FRS tests for paper **IV**, were grown in the plantation of Polli Horticultural Research Centre, Southern Estonia (58º06´N, 32º25´E). Two dark-rooted rhubarbs (´Victoria´ and seedling 303) and one light-rooted (´Ogres´) rhubarb were selected from among 16 different cultivars or seedlings, according to the content of hydroxyanthraquinones in their roots. The petioles of the same rhubarb cultivars or seedling were also selected for the test. Berries of chokeberry (selected from among three seedlings), blue honeysuckle (haskap berry) ´Tomitška´ (selected from among five cultivars) and blackcurrant ´Ben Alder´ (selected from among 37 cultivars) were all selected for the tests according to their highest content of anthocyanins. Leaves of blackcurrant ´Pamyati Vavilova´ were also included into this study. All of these plant materials were freeze dried, except rhubarb roots, using a VirTis AdVantage 2.0 EL freeze-dryer (SP Industries) and kept at a temperature of -40ºC until powdering. Roots of garden rhubarb cultivars and seedlings were washed, diced and dried at 50°C in a drying oven Binder FED101 (Binder GmbH) and kept at room temperature. All the dried plant materials were powdered with a blender (Stollar/Kinetix® Control) to a particle size diameter of ≤3 mm; the necessary fraction was obtained with an analytical sieve shaker AS300 control (Retsch GmbH). For the infusions, 1 g of each powder in duplicate was mixed with 20 ml of 20% and 96% aqueous ethanol. The mixtures were rotated on a Multi RS-60 Multirotator (Biosan) at 40 rpm for 24 h at room temperature, followed by centrifugation at 2594 *g* for 10 min on a Sigma 4-16KS (Sigma Laborzentrifugen GmbH) centrifuge. The supernatants were collected and further diluted by two, four and eight times for the estimation of AB and FRS properties, and for estimation of total polyphenol content (TPC). The main differences in the methodologies of the two papers (**III** and **IV**) were using decoction with buffered water in the comparison with 30% ethanol at room temperature (**III)**, and extraction at room temperature with 20% ethanol in the comparison with 96% ethanol (**IV).**

The total content of sugars and pH of the plant extracts in 20% ethanol (**IV**) were determined from Fourier transform near-infrared (FT-NIR) spectra, measured at controlled temperature, at a resolution of 4 cm^{-1} with the Bruker ALPHA ATR Platinum system (Bruker Optics GmbH) equipped with a diamond crystal and DTGS detector, with 128 scans for each sample in the range 375−4000 cm^{-1}. The background spectra were measured under the same conditions from water. The spectra were recorded by OPUS software version 7.5. (Bruker Optics GmbH) and analysed by using an OPUS software wine analysis wizard and calibration data.

4.5. Antibacterial properties of the studied samples

For antimicrobial properties testing in papers **III** and **IV** both Gram-negative and Gram-positive foodborne pathogenic bacteria together with well-known probiotics (**III**) and bacteria commonly used in sensitivity testing (**IV**) were selected, as shown in Table 2. Bacterial strains were obtained from the strain collections of the Estonian Veterinary and Food Laboratory and Department of Food Hygiene and Veterinary Public Health of Estonian University of Life Sciences. The bacterial cultures from the solid medium were subcultivated in liquid media. 1 ml loopful of bacterial mass was subcultivated in 10 ml of Mueller-Hinton broth (Oxoid) or agar, developed by de Man, Rogosa and Sharpe (MRS) broth (Oxoid) and by subsequent incubation at 37°C for 20 h (III). A certain amount of incubated bacterial suspension was mixed with 400 ml sterilized 45°C Mueller-Hinton agar (Oxoid), Columbia blood agar, Plate-count agar (Difco), Iso-Sensitest Agar (Oxoid) or diagnostic sensitivity test agar (DST) (Oxoid) (Table 2) to obtain a final density of 10^6 cfu/ml and then poured into Petri dishes for solidification at room temperature. Test-agar pH = 7 (Merck) and Test-agar pH = 8 (Merck) were used for testing *B. subtilis* and *K. rhizophila*, respectively. Control of the purity of the bacterial suspensions was carried out and the density of the bacterial suspensions was controlled. Wells were made into agar gel (6 mm in diameter) using a sterilized stainless steel borer and finally filled with 30 ml of abovementioned (Chapter 4.4) plant infusions with different dilutions: 1:10, 1:20, 1:40 and 1:80 (w/v) dilutions of infusions in buffered water or 30% ethanol (**III**), or 1:20, 1:40 1:80 and 1:160 dilutions of 20% ethanol and 96% ethanol infusions (**IV**) were used. 1,000 mg/l

chloramphenicol (LAB M) was used as a positive control, 30% ethanol and phosphate buffer (pH = 7) (**III**), and 20% with 96% ethanol (**IV**) were used as negative controls, respectively. After 24 h of incubation, the radii of the clear inhibition zone from the edge of the agar well were measured using a ruler to an accuracy of 0.5 mm and the antibacterial effect was calculated as a mean of duplicate tests. In AB properties studies, sodium nitrite was used as a control together with antibiotics (**III**). Nevertheless, $NaNO_2$ is probably more suitable for use as a control substance in food science, as it is used in the actual food systems for AB properties, additionally to food processing techniques, not the antibiotics. The microbiological analyses were not conducted by the author of this thesis, but by other members of the research group.

Table 2. The used media and incubation conditions for different bacteria (**III**, **IV**). The table is modified after Table 1 in **IV**

Bacterial culture	Agar-media	Incubation conditions
Gram negative		
Campylobacter jejuni ATCC 33291°•	Columbia blood agar (Oxoid) + 5% lysed horse blood (Oxoid)	42°C, 48 h, microaerobic
Salmonella Enteritidis ATCC 13076•	Mueller Hinton agar (Oxoid)	37°C, 48 h, aerobic
Escherichia coli NCCB 100282°•	Mueller Hinton agar (Oxoid)	37°C, 48 h, aerobic
Yersinia ruckeri NCIM 13282•	Plate-count agar (Difco), pH 6.5	30°C, 24-26 h, aerobic
Gram positive		
Listeria monocytogenes ATCC 13929°•	Mueller Hinton agar (Oxoid)	37°C, 48 h, aerobic
Bacillus cereus ATCC 11778•	Iso-Sensitest Agar (Oxoid), pH 6 + 625 μg/l CAP	30°C, 24-26 h, aerobic
Kocuria rhizophila ATCC 9341°•	Iso-Sensitest Agar (Oxoid), pH 8	30-37°C, 24-26 h, aerobic
Bacillus subtilis BGA°•	Plate-count agar (Difco), pH 8	30-37°C, 24-26 h, aerobic
Bacillus pumilus CV 607•	DST-agar (Oxoid), pH 7	37°C, 24-26 h, aerobic
Bifidobacterium bifidum Bb12°	Mueller Hinton agar (Oxoid), pH 7	37°C, 20 h, anaerobic
Lactobacillus acidophilus ATCC 4356°	Mueller Hinton agar (Oxoid), pH 7	37°C, 20 h, microaerobic

○ – The bacterial strain used in the paper III
● – The bacterial strain used in the paper IV

4.6. Free radical scavenging properties

The rhubarb roots and petioles, together with blackcurrant berries and leaves and other plant materials: bilberry, blue honeysuckle, sea buckthorn and tomato fruits (**III**), blue honeysuckle and chokeberry berries (**IV**) were tested for FRS properties. The main differences in the methodologies of the two papers were as follows: In paper **III**, the FRS properties of the plant infusions were compared with the same properties of ascorbic acid. In paper **IV**, the most common polyphenolic compound, found in many plants − quercetin 3-rutinoside (rutin) was used as the FRS capacity reference. For HPLC analysis 1:20 (w/v) dilutions, for antioxidant efficiency 1:10 (**III**), 1:20, 1:40, 1:80 (**III**, **IV**) and 1:160 (**IV**) (w/v) dilutions were used.

The free radical scavenging capacity was determined using the stable free radical DPPH decolourization assay at an absorption maximum 515 nm using an AnalyticJena Specord 200 spectrophotometer (AnalyticJena AG, Germany) with WinASPECT Software package. DPPH methanol solution (6.02×10^{-5} M *i.e.* 23.7 mg/l) was made and kept covered from light in a refrigerator. Hundred microliters of tested infusion (A) or (B) was mixed with 3,900 ml DPPH solution in a spectrophotometric cuvette and the absorbance was recorded immediately after mixing and after every 10 min during a 60 min period until a steady state of the reaction was registered (**III**, **IV**). The reference cuvette (blank), contained aqueous phosphate buffer or 30% ethanol (**III**) or 20% ethanol or 96% ethanol (**IV**), respectively. The FRS capacity was expressed as reduced DPPH% (**III**) or as rutin equivalent (g/l) (**IV**). The method used was a modification of the methods described by Huang *et al.* (2005) and Helmja *et al.* (2008). All the FRS assays were performed in duplicate and as a comparison either solution of antioxidant ascorbic acid in buffered water or in 30% ethanol, at the concentration of 1 mg/ml and 10 mg/ml (**III**), or the rutin solution at different concentrations were used (**IV**). The AO efficiency (AO%), showing the reduced (%) of DPPH stable free radical, was calculated using the formula:

$$AO\% = [A_{0(DPPH)}-A_{f(sample)}]/A_{0(DPPH)}\times100,$$

where A_0 is the absorbance of DPPH, and A_f is the absorbance of the reaction sample (DPPH, with the addition of the plant infusion) in the final state of the reaction, after 60 min (Anton *et al.*, 2014).

4.7. Statistical analysis

In paper **III**, principal component analysis (PCA) and Spearman's rank correlation analysis were carried out, using R statistical software (version 2.14.1). Calibration curves of pure standards were estimated using linear regression analysis in Microsoft Excel. The results of statistical analysis were left out from the paper **III**, due to the small sample size (duplicate measurements of each sample), unfortunately the respective correction in the paper's paragraph "Materials and methods", was not made. But later, the statistical results (**III**) were reconsidered and presented in the thesis.

In paper **IV**, MS Excel 2013 software was used to evaluate linear correlations (Pearson) between different chemical properties and in vitro properties of the plant infusions. The correlation was considered strong if $|r| \geq 0.65$, moderate if $|r| > 0.40$ or weak if $|r| \leq 0.4$. Due to the small sample size (duplicate measurements of each sample) the p values are not calculable and all results are only descriptive, indicating potential patterns, relationships and differences in the present thesis.

5. RESULTS

5.1. Polyphenols of garden rhubarb samples

Different derivatives of resveratrol, piceatannol, rhapontigenin, deoxyrhapontigenin, chrysophanol, emodin, torachrysone, pterostilbene and aloe-emodin were identified in rhubarb roots (Table 1, **I**). The petioles of rhubarb additionally contained several flavonols, derivatives of quercetin and myricetin (Table 2, **I**), identified by comparing their MS^2 spectra with respective spectra of the commercial standards of quercetin or myricetin. Results show that roots of rhubarb contain a wide variety of hydroxystilbene glycosides, which makes this plant interesting from the medicinal point of view. In paper **IV**, several different seedlings of *R. rhaponticum* were analysed and the most abundant profile of polyphenols is presented in Fig. 7 and Table 3, extracted both with 20% and 96% of ethanol, with each solvent producing a somewhat different polyphenolic profile. The additional compounds that were extractable with 96% ethanol compared to 20% ethanol are marked with asterisks in Table 3.

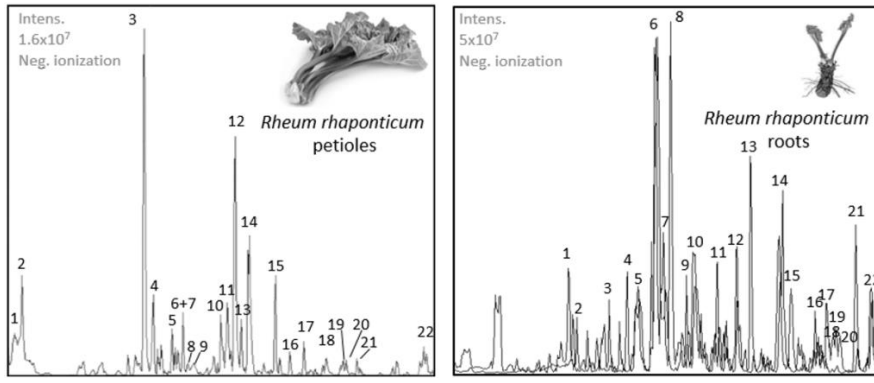


Figure 7. The base peak chromatograms (BPC) of rhubarb petioles in 20% ethanol and root extracts in both 20% and 96% ethanol (BPC overlapped), analysed with negative ionization mode, the peak numbers (by the axis x) refer to chronological retention order of the compounds, listed in Table 3 (**IV**)

Table 3. The compounds of *Rheum rhaponticum* L. petioles and roots, the peak numbers refer to the compounds on Fig. **7** in order of increasing retention time (**IV**)

Peak	***Rheum rhaponticum* petioles**	**[M-H]-/ fragments**	**[M+H]+/ fragments**
1	Citric acid	191/111;173	
2	Gallic acid	331/169	
3	Catechin	289/245	
4	Paracoumaric acid-glucoside	325/145	
5	Ferulic acid glucoside	355/193	
6	Epicatechin	289/245	
7	Myricetin glucuronide	493/317;179	
8	Cyanidin-3-*O*-glucoside		449/287
9	Cyanidin-3-*O*-rutinoside		595/287
10	Myricetin rutinoside	625/317	
11	Taxifolin glucoside	465/303;151	
12	Epigallocatechin gallate or gallocatechin gallate	441/289	
13	Myricetin-rhamnoside	463/317	
14	Rutin	609/301	
15	Quercetin glucuronide	477/301	
16	Quercetin rhamnoside	447/301	
17	Kaempferol rutinoside	593/285	
18	Phloridzin	435/273	
19	Myricetin glucoside glucuronide	479/316	
20	Deoxyrhapontin	403/241	
21	Quercetin glucoside	463/301	
	***Rheum rhaponticum* roots**		
1	Procyanidin B1	577/407;289	
2	Catechin	289/245	
3	Epicatechin	289/245	
4	Piceatannol-*O*-glucoside 1	405/243	
5	Resveratrol-*O*-glucoside 1	389/227	
6	Piceatannol-*O*-glucoside 2	405/243	
7	Resveratrol-O-glucoside 2 (Piceid)	389/227	
8	Piceatannol	243/225	
9	Rhapontigenin-*O*-glucoside 1	419/257	
10	Rhapontigenin-*O*-glucoside 2	419/257	
11	Rhapontigenin-*O*-glucoside 3	419/257	
12*	Aloe-emodin-*O*-glucoside	431/269	
13	Rhapontigenin	257/241	
14*	Torachryson-*O*-glucoside	407/245	
15*	Emodin-*O*-glucoside	431/269	
16	Deoxyrhapontigenin-*O*-galloylglucosde	555/313;169	
17*	Torachrysone-*O*-acetylglucoside	449/245	
18*	Chrysophanol-*O*-glucoside	415/253	
19*	Rhein-*O*-glucoside	445/283	
20*	Chrysophanol-*O*-acetylglucoside	457/253	
21	Deoxyrhapontigenin	241/226	
22	Resveratrol dimer	453/453	

*- the compound is better extractable with higher concentration of the organic solvent

5.2. Polyphenols of blackcurrant berries and leaves

The results of the HPLC-UV-Vis-MS/MS analysis of the blackcurrant leaves (**II**) are presented in Fig. 8. The analysis was conducted using polyphenol standards (2-catechin, 3-chlorogenic acid, a-quercetin galactoside, 6-quercetin glucoside, b-myricetin, 8-quercitrin, c-quercetin, d-kaempherol), previously known as present in the blackcurrant leaves (Bisset, 1994; ESCOP, 2003) or in other leaves of pharmacological value (Harris *et al.*, 2007). Some of the standard compounds were present in the blackcurrant leaves (peaks 2, 3, 6 and 8). The anthocyanins, found in BC berries (**II**), are shown in Fig. 9. Compounds that were not identical with any of the used standards were identified using their ionic weight and MS^2 collision fragments pattern. The quantitative analysis was performed using the peak areas under MS extracted ion chromatograms.

In study **IV**, the major peaks on base peak chromatograms (BPC) of blackcurrant leaves and berries were identified using the MS^2 collision fragment patterns and retention times of the polyphenol standards. The following compounds were found in the leaves: catechin gallate, chlorogenic acid I, dihydro ferulic acid rhamnoside, chlorogenic acid II, ferulic acid derivative, coumaryl quinic acid, coumaroylquinic acid pentoside, myricetin-glucoside, quercetin-3-rutinoside syn. rutin, quercetin glucoside, quercetin acetylglucoside, kaempferol rutinoside, kaempferol-3-*O*-glucoside, kaempferol acetylglucoside, isorhamnetin acetylglucoside and chrysophanol glucoside. In the berries of blackcurrant, the following compounds were identified: chlorogenic acid, caffeic acid-*O*-glucoside, coumaryl quinic acid, delphinidin-3-*O*-glucoside, delphinidin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside, myricetin-*O*-glucoside and rutin (Fig. 10 and Table 4). This polyphenolic profile is comparable with that of Vagiri *et al.* (2012). However, since then, Tian *et al.* (2019) have identified more compounds, especially several different anthocyanins, in blackcurrant berries, which may be the result of a more precise analysis method or some other factors of the sample analysis.

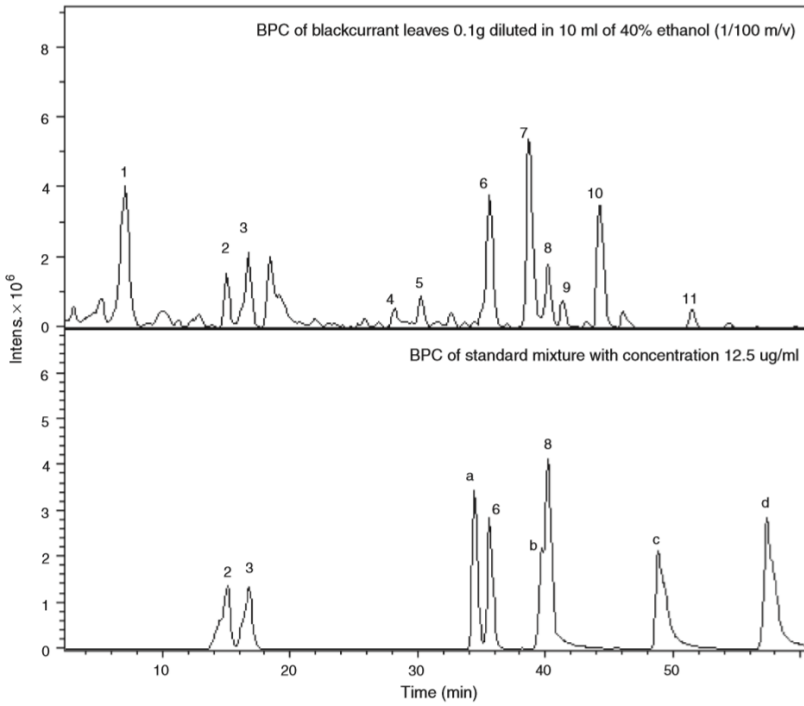


Figure 8. The base peak chromatograms (BPC) of leaves (above) and a standard mixture of polyphenols that can be found in many plants (below). The standard compounds, not found in blackcurrant leaves (**II**), are marked with letters. 1. gallocatechin; 2. epigallocatechin; 3. chlorogenic acid; 4. unidentified compound with $[M\text{-}H]^-$ = 383; 5. myricetin glucoside; 6. quercetin-*O*-glucoside; 7. quercetin malonylhexoside; 8. mixture of kaempferol glucoside, quercetin rutinoside and quercetin acetylglucoside; 9. mixture of isorhamnetin rutinoside and an unidentified glucoside with $[M\text{-}H]^-$ = 373; 10. kaempferol malonylhexoside; 11. isorhamnetin-*O*-glucoside (**II**)

The qualitative polyphenolic composition of the berries and leaves of blackcurrant as well as of rhubarb roots and petioles identified in the course of the present study is evidently not a complete and final list of the polyphenolic compounds, to be found in these plant tissues. As mentioned in the introduction, the profile of metabolites such as polyphenols in the plant tissue may vary due to many factors. The polyphenolic composition at a particular stage of plant maturity may differ from that at another time point. In addition, different analysis methods may enable identification, to some extent, of different compounds. In addition, the author is aware of the limitations due to ones´ knowledge and the analysis equipment used, at the time of the analyses.

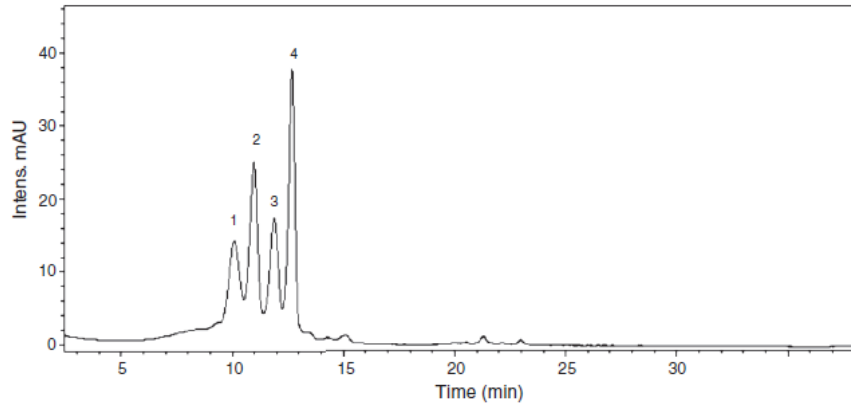


Figure 9. The UV-chromatogram of blackcurrant fruit anthocyanins at 510 nm. 1) delphinidin-*O*-glucoside, 2) delphinidin 3-*O*-rutinoside, 3) cyanidin-*O*-glucoside, 4) cyanidin 3-*O*-rutinoside (**II**)

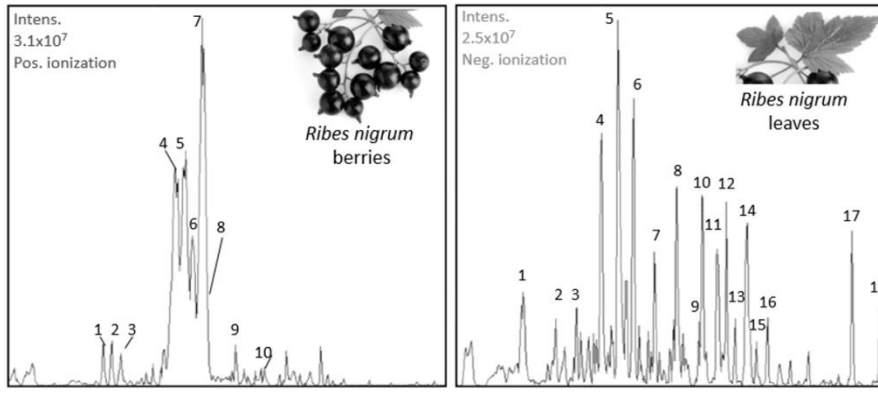


Figure 10. The base peak chromatograms of blackcurrant berry and leaf extracts in 20% of ethanol (**IV**), the peak numbers (by the axis x) refer to the chronological retention order of the compounds, listed in Table 4

Table 4. The qualitative composition of the blackcurrants 20% ethanol extracts analysed with negative and/or positive ion mode. The peak numbers refer to Fig. 10, listed in order of retention times (**IV**)

Peak	*Ribes nigrum* berries	[M-H]-/ fragments	[M+H]+/ fragments
1	Chlorogenic acid	353/191;179	
2	Caffeic acid-*O*-glucoside	345 (341)/179;161	
3	Coumaryl quinic acid	337/191	
4	Delphinidin-3-*O*-glucoside		465/303
5	Delphinidin-3-*O*-rutinoside		611/465;303
6	Cyanidin-3-*O*-glucoside		449/287
7	Cyanidin-3-*O*-rutinoside		595/287
8	Isorhamnetin-3-*O*-rutinoside		625/317
9	Myricetin-*O*-glucoside		481/319
10	Rutin	609/301	611/303
	Ribes nigrum leaves		
1	Catechin gallate	305/179;219;261;137	
2	Chlorogenic acid I	353/191;179	
3	Dihydro ferulic acid rhamnoside	341/195;163;129	
4	Chlorogenic acid II	353/191	
5	Ferulic acid derivative	399/193;301	
6	Coumaryl quinic acid	337/191	
7	Coumaroylquinic acid pentoside	675/337;191	
8	Myricetin-glucoside	479/317;179;151	
9	Quercetin-3-rutinoside syn. rutin	609/301	
10	Quercetin glucoside	463/301	
11	Quercetin acetylglucoside	505/301	
12	Kaempferol rutinoside	593/285	
13	Kaempferol-3-*O*-glucoside	447/285	
14	Kaempferol acetylglucoside	489/285	
15	Isorhamnetin acetylglucoside	519/315	
16	Chrysophanol glucoside	415/373;355	

5.3. Antibacterial properties

The findings (**III**, **IV**) showed that plant ethanol infusions have greater AB effect than water infusions. Study **III** showed that some plant infusions, *e.g.* of blue honeysuckle, sea buckthorn and garden rhubarb petioles can have an AB effect against food-borne bacteria *L. monocytogenes*, *E. coli* or *C. jejuni* without strong inhibition towards probiotic bacteria *B. bifidum* (Table 2 in **III**). Among the studied plants (**III**), 30% ethanol infusions of bilberry and sea buckthorn had the greatest AB effect against *E. coli*. Rhubarb root, blue honeysuckle and

blackcurrant infusions showed strong or moderate AB effect (**III**). The tomato infusions showed very limited AB effect in study **III**.

In study **III**, sodium nitrite salt at 2.5% and 5% in buffered water solutions was used in comparison with plant materials. However, the used concentrations of sodium nitrite did not show any AB effect against tested bacteria. Our results can be explained by the bacterial species selection or by the use of agar-well method instead of nutrient broth media, which probably can more properly imitate the action of nitrite in food products. In the following AB studies with meat matrix, corrections were made in the sodium nitrite concentration used (Anton *et al.,* 2019).

Among all the tested plants, the roots of rhubarb showed the strongest AB effect against tested bacteria in paper **III** (Fig. 11), and in the paper **IV** the effect was reestablished, but also 96% ethanol infusions of rhubarb petioles and blackcurrant berries showed an AB effect against all the tested bacterial species (Fig. 12).

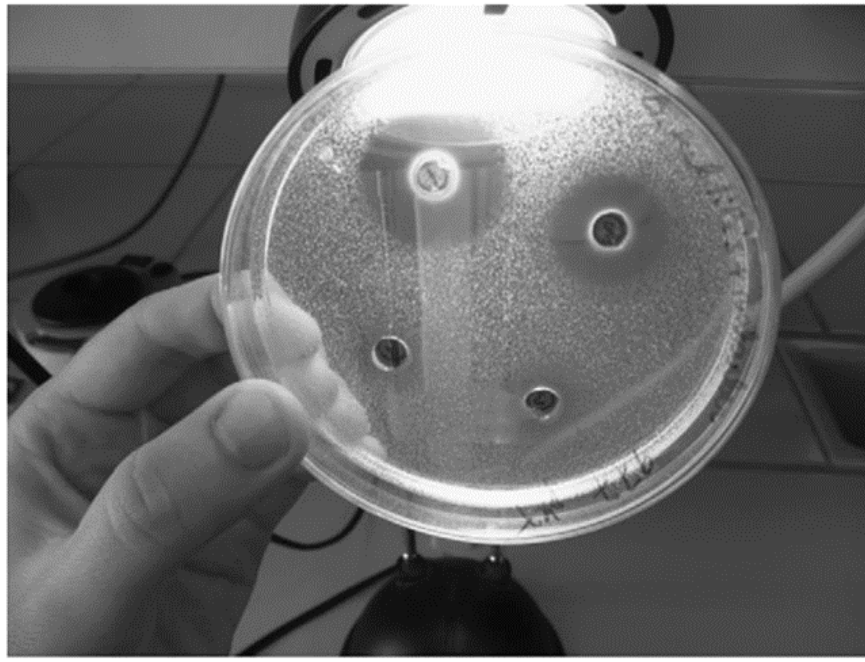


Figure 11. The influence of the buffered water infusions of garden rhubarb root: 1:10 (top left); 1:20 (top right); 1:40 (bottom right) and 1:80 (bottom left) (w/v) to the growth of *Bacillus subtilis* (**III**)

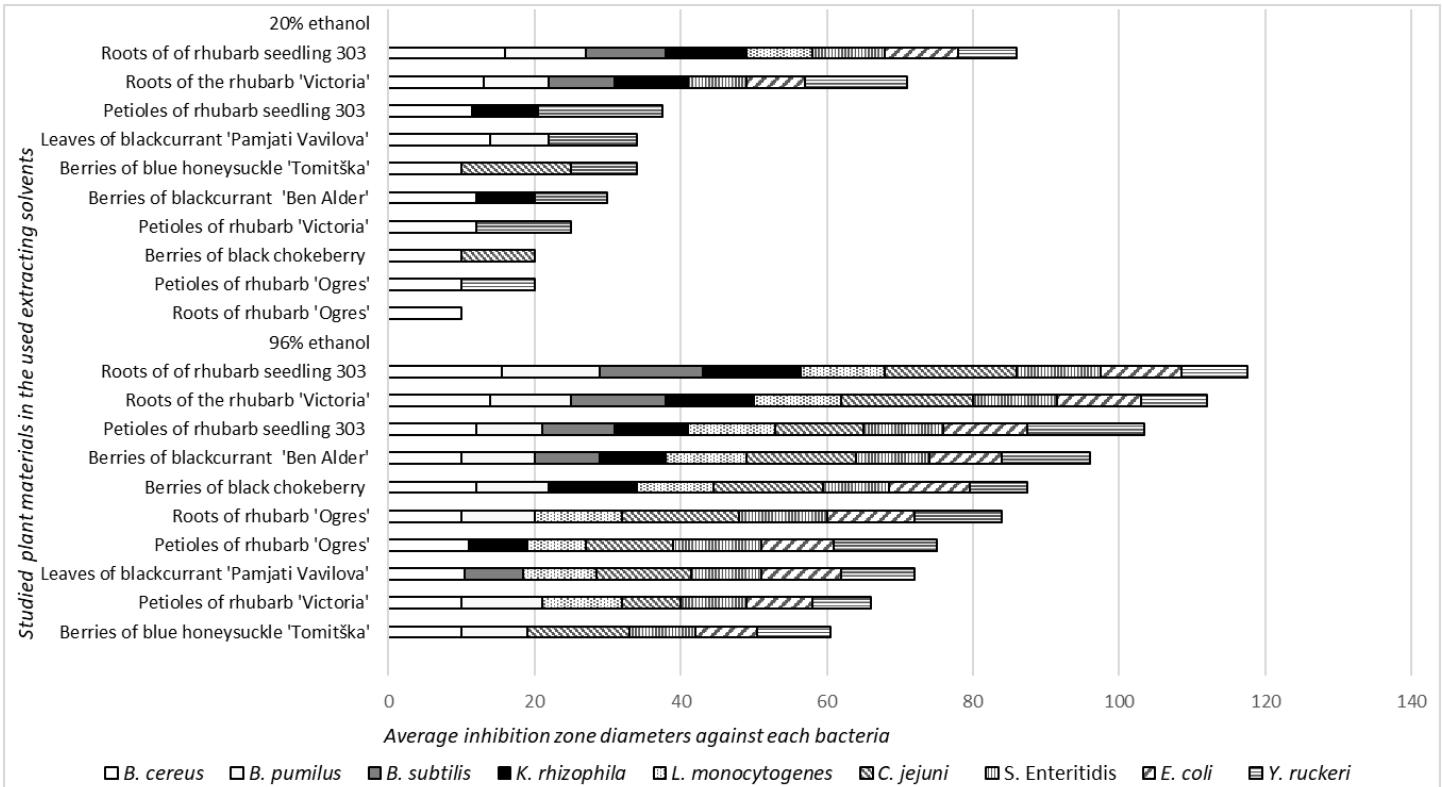


Figure 12. The bars, on the axis x, represent the summarized average bacterial growth inhibition zone diameters (mm) of the 1:20, w/v dilutions of the plant infusions in 20% and 96% ethanol against each bacteria, listed in descending order of summarized antibacterial properties (**IV**)

In paper **IV**, *in vitro* AB properties were estimated against both Gram+ and Gram− bacteria (Table 2, Fig. 12). In the case of 20% ethanol–plant infusions, Gram− bacteria were less susceptible than Gram+ bacteria. The strongest AB effect of tested plants´ 96% ethanol infusions was against *C. jejuni*, which is a Gram− micro-aerobic bacterium, and against *B. cereus*, which is a Gram+ aerobic bacterium. An important find of the present study was that growth of Gram+ foodborne pathogenic bacteria *L. monocytogenes* and *B. cereus* as well as Gram− pathogens *C. jejuni*, *S.* Enteritidis and *E. coli* were inhibited by the 96% ethanol infusions of the roots and petioles of rhubarb (both seedling 303 and ´Victoria´). This makes rhubarb a promising candidate for use as the source of natural ABs in food. Blackcurrant berries also had an AB effect against all of the tested bacterial species in paper **IV**. It is notable that *L. monocytogenes*, which is known as a relatively resistant bacterium to different environmental factors, was susceptible to nine out of ten tested plants´ 96% ethanol infusions (**IV**). With some bacterial species, AB properties depended on the total content of polyphenols and only with *C. jejuni,* on the content of anthocyanins, both in 20% and 96% ethanol infusions (**IV**).

5.4. Free radical scavenging properties

In paper **III**, all the studied plant infusions 1:10 w/v, except those of the garden rhubarb roots, prepared in the buffered heated water, showed higher FRS capacity than the solution of ascorbic acid at the concentration of 1 mg/ml. Heating the sample, before analysis, resulted in remarkably lower ascorbic acid content compared to the solutions, prepared in 30% ethanol at room temperature (Table 5). The order of the studied plant infusions in buffered water, starting from the highest FRS effect, was blue honeysuckle, blackcurrant, ascorbic acid (10 mg/ml), sea buckthorn, rhubarb petioles, bilberry, tomato, ascorbic acid (1 mg/ml) and rhubarb roots (Table 5). In 30% ethanol infusion, however, the order of antioxidative effect of the studied plant materials was different. Starting with the highest FRS effect, the antioxidativities decreased in the order: ascorbic acid (10 mg/ml), rhubarb petioles, blackcurrant berries, bilberry, rhubarb root, blue honeysuckle, ascorbic acid (1 mg/ml), sea buckthorn, tomato (Table 5). The FRS effect of the blue-coloured berries at higher concentrations was difficult to estimate since the DPPH absorbs light at the same wavelength (515 nm) as the anthocyanins (Chaovanalikit *et al.*, 2004). Nevertheless, at the dilution

rate of 1:40 to 1:160 (w/v), the tested plant material did not interfere with the colour loss of DPPH during the reaction, as presented in Table 5. Results showed that the antioxidant effect of the analysed plants was different, with different extraction solvent. The antioxidant properties were positively correlated with the anthocyanin ($r = 0.56$) and ascorbic acid content ($r = 0.94$) in the heated water infusion, but weak correlations were established between the same parameters in the ethanol infusion, ($r = 0.41$) and ($r < 0.1$) respectively (**III**). The results of the PCA of the relations of plant materials and their measured chemical parameters are presented on Fig. 13 (in heated buffered water) and Fig. 14 (in 30% ethanol). These results generalise the correlation patterns studied with Spearman's rank correlation analysis (**III**).

In paper **IV**, where 20% and 96% ethanol were used as extracting solvents, the correlations between ascorbic acid and FRS were respectively weak to moderate ($r = 0.4-0.64$). In paper **IV**, chokeberry berries, characterized by the highest content of anthocyanins, both in 20% and 96% aqueous ethanol infusions, showed the greatest FRS properties, expressed by DPPH free radical scavenging ability. Chokeberry berries were followed by 20% aqueous ethanol infusion of blackcurrant berries with the lowest total polyphenols and total anthocyanins content among the berries. Blue honeysuckle, with the highest total polyphenol content among berries and dark rhubarb roots (**IV**) with the absolutely highest TPC, were also efficient. The darkness of rhubarb roots is related to their content of anthraquinones. The AO properties of chokeberry, blackcurrant berries and blue honeysuckle berries were, however, very similar (**IV**). Summary FRS of both 20% ($r = 0.65$) and 96% ($r = 0.47$) ethanol infusions of plants was positively correlated with the content of anthocyanins, which is in the agreement with the results of Heinonen (2007) and Shih *et al.* (2007). In addition, FRS properties of plant infusions had moderate positive correlation with the content of citric and ascorbic acids (Fig. 4 in **IV**).

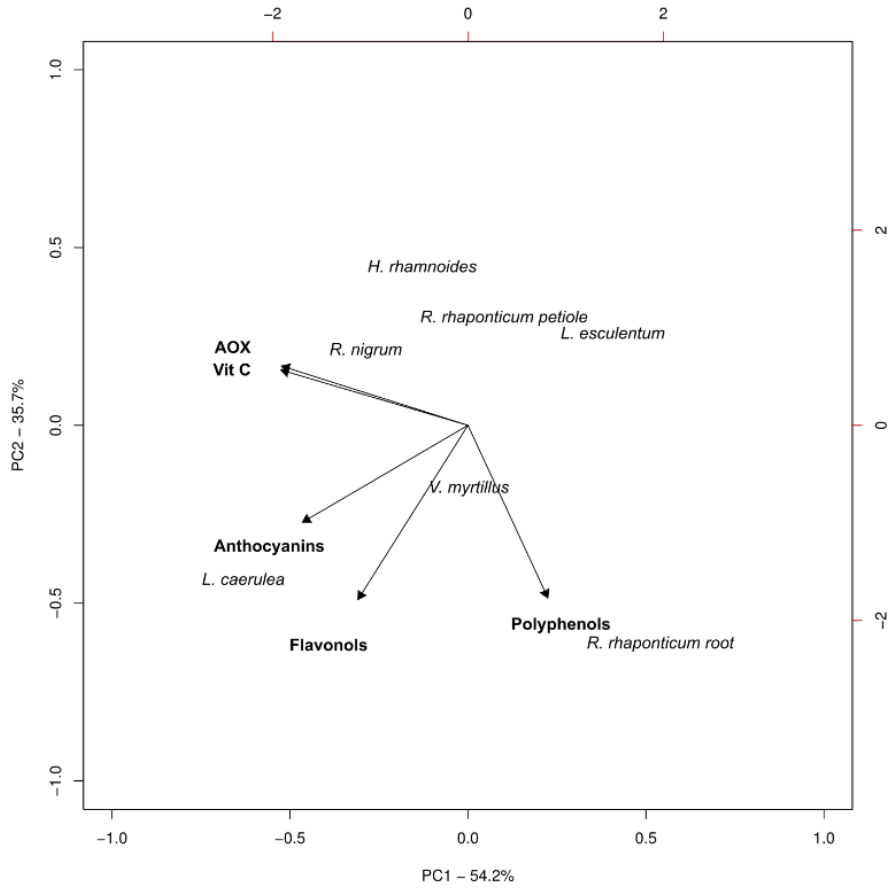


Figure 13. The results of principal component analysis of the chemical properties of plant materials in heated buffered water (extracting solvent A) (**III**), AOX−antioxidative efficiency (the reduced amount (%) of DPPH)

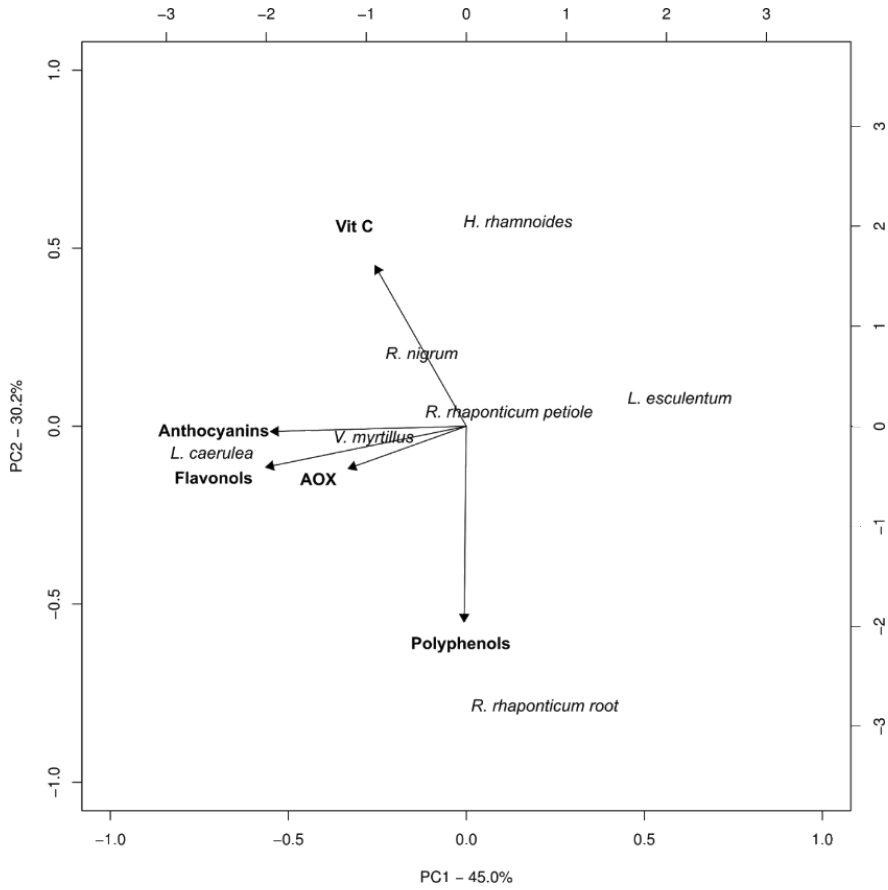


Figure 14. The results of principal component analysis of the chemical properties of plant materials in 30% ethanol (extracting solvent B) (**III**), AOX−antioxidative efficiency (the reduced amount (%) of DPPH)

Table 5. The average values of two parallel measurements of free radical scavenging efficiency of the studied samples (AO%), together with the content of vitamin C (Vit-C), total anthocyanins (Anth.) and total polyphenols (TPC) (**III**)

Test material	AO% A	AO% B	Vit-C (mg/ml) A	Vit-C (mg/ml) B	Anth. (mg/ml) A	Anth. (mg/ml) B	TPC (mg/ml) A	TPC (mg/ml) B
Tomato	37	49	0.02	0.03	0.00	0.00	0.5	0.3
Bilberry berries	37	91	0.07	0.15	0.20	0.18	1.3	1.7
Sea buckthorn berries	74	74	0.13	0.32	0.00	0.00	0.2	0.1
Blackcurrant berries	84	95	0.12	0.21	0.13	0.11	0.8	0.7
Rhubarb root	21	87	0.02	0.03	0.00	0.00	7.7	8.5
Rhubarb petiole	48	98	0.10	0.13	0.01	0.01	0.6	0.9
Blue honeysuckle berries	86	84	0.14	0.19	0.35	0.36	2.4	2.4

AO% − antioxidative efficiency = free radical scavenging efficiency (FRS), the reduced amount (%) of DPPH

A − infusions, made into phosphate buffer (1:40 w/v) and heated at 95°C for 10 min

B − infusion, obtained from infusing plant material in 30% ethanol (1:40 w/v) for 24 hours at room temperature, with periodical shaking

6. DISCUSSION

6.1. Polyphenols of rhubarb roots and petioles

The polyphenolic composition and quantity of different compounds in different garden rhubarb cultivars may differ (Rumpunen and Henriksen, 1999). Nevertheless, there are some major groups of compounds that are generally present in roots and petioles like *trans*-stilbenes (resveratrol, piceatannol, rhapontigenin, deoxyrhapontigenin and their *O*-glucosides), anthraquinones (emodin, chrysophanol, rhein and their glucosides) and naphthalene derivative torachrysone glucoside (Kolodziejczyk-Czepas *et al.*, 2020). These compounds were identified in the rhubarb samples in paper **I**. In the petioles, the major flavonols are catechin, epicatechin, epigallocatechin gallate or gallocatechin gallate, myricetin-*O*-rhamnoside, quercetin-*O*-rutinoside (rutin), quercetin-*O*-glucuronide, quercetin-*O*-glucoside, quercetin-*O*-rhamnoside and quercetin (Komatsu *et al.*, 2006). The same compounds were identified in the studies for papers **I** and **IV** (Table 3). When anthocyanins are present in the petioles (depending on the cultivar or genotype), these are cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside (Takeoka *et al.*, 2013). Anthocyanins were identified in the rhubarb petioles in the course of the studies for paper **IV**, in the positive ionisation mode with HPLC-both with MS/MS and UV-Vis detector. Takeoka *et al.* (2013) determined the total content of anthocyanins in rhubarb petioles 20−341 mg in 100 g DW; Rumpunen and Henriksen (1999) analysed 71 different genotypes of culinary rhubarbs (*Rheum* spp.) and found anthocyanin content in the rhubarb petiole juice 0−154 mg/kg in FW. These differences may result in different *in vitro* properties of the cultivars of garden rhubarb.

6.2. Polyphenols of blackcurrant berries and leaves

The identified polyphenolic profile of BC berries and leaves in the present thesis (**II**) is comparable with the analysis results of Vagiri *et al.* (2012). However, since then, Tian *et al.* (2019) identified more compounds, especially several different anthocyanins: pelargonidin 3-*O*-glucoside, pelargonidin 3-*O*-rutinoside, peonidin 3-*O*-glucoside, peonidin 3-*O*-rutinoside, malvidin 3-*O*-glucoside, malvidin 3-*O*-rutinoside, delphinidin 3-*O*-(6"-coumaroyl)-glucoside, cyanidin 3-*O*-(6"-coumaroyl)-glucoside in blackcurrant berries. This may result from more

precise analysis equipment and method or some other factors of the sample analysis. The latter indicates that qualitative analysis of a plant material is never complete and final as the analytical tools improve with time and the state of the plant tissue maturity is difficult to estimate and equalize among different studies.

Blackcurrant berries contain, besides polyphenols, organic acids: citric acid, malic acid, tartaric acid, fumaric acid and shikimic acid, on the average: 1.26; 0.73; 0.14; 0.95 and 2.65 mmol/100 g FW, respectively (Mikulic-Petkovsek *et al.*, 2012) and ascorbic acid 148–310 mg/100 g FW (Vagiri *et al.*, 2013). These compounds may also contribute to the *in vitro* properties of the berries, similarly to rhubarb or any other plant material, where different organic acids are present. In BC leaves or buds, additionally to the different polyphenolics and organic acids, volatile compounds (Dvaranauskaite *et al.*, 2008) may give input to the *in vitro* properties.

6.3. Antibacterial properties of the studied samples

In the papers **III** and **IV** was revealed that plant ethanol infusions have a greater AB effect than water infusions, as they contain more hydrophobic compounds. These results are in agreement with Sebiomo *et al.* (2011). Li *et al.* (2016), who showed that hydrophobic emodin may destroy cell membrane integrity by influencing the conformation of membrane proteins and thereby increase membrane permeability. This mechanism can explain the highest AB effect of rhubarb root 96% ethanol infusion against Gram− bacteria in the present study, as in addition to emodin, it contains several other relatively hydrophobic compounds. Study **III** showed that some plant infusions, *e.g.* of blue honeysuckle, sea buckthorn and garden rhubarb petioles can have an AB effect against food-borne bacteria *L. monocytogenes*, *E. coli* or *C. jejuni* without strong inhibition towards probiotic bacteria, especially *B. bifidum*. Therefore, selected plant infusions in combination with the studied probiotic bacteria could be tested in food processing as potential antioxidants, antimicrobials, and functional ingredients (Table 2, **III**). In general, the AB properties of the studied plant materials were more strongly correlated with the content of total polyphenols than with the other measured parameters. On the other hand, the highest FRS effect was determined in plant materials containing anthocyanins, citric acid and ascorbic acid. There was no correlation between AB and FRS

properties of the studied plant infusions, indicating that different compounds may be involved in FRS properties compared to AB properties. The used amounts of sodium nitrite (**III**) did not show any AB effect against tested bacteria. Our results can be explained either by bacterial species selection or by the use of the agar-well method instead of nutrient broth media, which probably can better imitate the action of nitrite in food products (Xi *et al.*, 2011). In the following AB studies with meat matrix the sodium nitrite concentration used was amended (Anton *et al.*, 2019).

An important finding of the study **IV** was, that growth of Gram+ foodborne pathogenic bacteria *L. monocytogenes* and *B. cereus* as well as Gram− pathogens *C. jejuni*, *S.* Enteritidis and *E. coli* were inhibited by the 96% ethanol infusions of the roots and petioles of rhubarb (both seedling 303 and ´Victoria´). This makes rhubarb a promising candidate for use as the source of natural ABs in food. The same effect was achieved by the blackcurrant berries. It is notable that *L. monocytogenes*, which is known as a relatively resistant bacterium to different environmental factors, was susceptible to nine out of ten tested 96% ethanol infusions (**IV**). Polyphenolic composition and hence, AB and AO properties of the plant extracts, depended on several factors. With some bacterial species, AB properties depended on the total content of polyphenols and only with *C. jejuni,* on the content of anthocyanins, both in 20% and 96% ethanol infusions (**IV**). The AO properties depended on the content of vitamin C, citric acid, anthocyanins and some, but not equally by all polyphenolic compounds in the plants, as can be concluded from the weak positive correlation between AO and TPC (**IV**).

6.4. Free radical scavenging properties

Sample heating prior to analysis resulted in remarkably lower ascorbic acid content compared to the solutions, prepared in 30% ethanol at room temperature (Table 5). This concurs with the results of previous studies, indicating high susceptibility of ascorbic acid to a rise in temperature (Zhang and Hamauzu, 2004). It may be one of the reasons why the FRS properties of the buffered water infusions were weaker than those of ethanol infusions (Table 5). Other reasons could be that the buffer reduced the FRS effect of ascorbic acid or that ethanol infusions contained more hydrophobic compounds, which had stronger FRS properties than water soluble compounds. In plant material, the

FRS properties may depend on the presence of the water-soluble ascorbic acid as well as the semi polar polyphenolic compounds or hydrophobic compounds (Zhang and Hamauzu, 2004). Later studies were performed with unaltered plant infusions (**IV**) or using plant material directly in the raw or cooked food matrix (Anton *et al.*, 2019). Previous studies have additionally shown, that FRS properties of plants are dependent on the total content of the polyphenols (Villano, *et al.*, 2007), especially of the anthocyanins (Chaovanalikit *et al.*, 2004; Viljanen *et al.*, 2005; Paśko *et al.*, 2009). The highest content of anthocyanins, within the present thesis, was found in blue honeysuckle (**III**) and in chokeberry (**IV**). Starting with the highest, the FRS effect of 30% ethanol infusions decreased in the order: ascorbic acid (10 mg/ml), rhubarb petioles, blackcurrant berries, bilberry, rhubarb root, blue honeysuckle, ascorbic acid (1 mg/ml), sea buckthorn, tomato (**III**). The results indicate that our presumption, that anthocyanins contribute to the FRS properties the most, is not entirely confirmed, as rhubarb petioles contain very little anthocyanins, even BC berries, that showed strong FRS properties, contain less anthocyanins than blue honeysuckle or bilberries. However, in the next study (**IV**) the FRS properties were listed in decreasing order: chokeberry berries, blackcurrant berries, blue honeysuckle berries and then rhubarb petioles. Again, additionally to anthocyanins some other compounds must have influenced the FRS properties, if we look at the anthocyanin content of BC berries, compared to chokeberry and blue honeysuckle berries. Probably, AO properties of blackcurrant berries are primarily dependent on hydrophilic compounds such as ascorbic acid, (Vagiri *et al.*, 2013) and additionally on semi-polar anthocyanins (Heinonen, 2007; Caprioli *et al.*, 2016) and flavon-3-ols – particularly rutin (Afanas'ev *et al.*, 1989) – all of which are good antioxidants. The highest content of anthocyanins was found in blue honeysuckle (**III**) and in chokeberry (**IV**). The highest content of total polyphenols was found in rhubarb root (**III**, **IV**), which regardless, had the lowest FRS capacity in the water infusion (**III**). This result could be explained with the fact, that the main constituents of the garden rhubarb root infusion are *trans*-hydroxystilbenes, mostly characterized by the modest antioxidativities (Helmja *et al.*, 2008). AO of rhubarb roots is probably more dependent on relatively hydrophobic constituents such as the hydroxyanthraquinones: emodin, aloe emodin and chrysophanol, as well as resveratrol dimers and trimers (Yen *et al.*, 2000), which are extractable from the plant matrix with a less polar solvent such as 96% ethanol. This can be concluded from the stronger

AO properties of 96% ethanol extracts of rhubarb roots compared to 20% ethanol extracts (**IV**) or water extracts (**III**). The berries with higher anthocyanin content had higher FRS properties, which is in accordance with the results of Viljanen *et al.* (2005), Paśko *et al.* (2009) and Tian *et al.* (2018).

The purpose of the present study was to reveal the overall FRS and AB properties of some food plants. But, in future studies it will be necessary to determine, which polyphenolic or other compounds in plant material have stronger FRS or AB properties. Furthermore, the plant materials have to be standardized for these compounds to have similar effects on every occasion when they are used in foods.

The AO properties of some polyphenol standard compounds and ascorbic acid in the comparison, in descending order, according to Villano *et al.* (2007) and Csepregi *et al.* (2016) would be: cyanidin ~ delphinidin ~ quercetin ~ myricetin > catechin > gallic acid > rutin > malvidin ~ pelargonidin > ascorbic acid > *trans*-resveratrol > *trans*-rhapontin > emodin.

7. CONCLUSIONS AND RECOMMENDATIONS

1. The AB and FRS properties of the studied plant materials were not in strong positive correlation, thus different compounds may be involved in the FRS effect compared to the AB effect. Therefore, a combination of plants with strong AB and plants with strong FRS effect should be used in combination to achieve the best results in a food matrix. The combination of rhubarb petioles, berries of black chokeberry, blackcurrant, blue honeysuckle or some other ascorbic acid- or anthocyanin-rich berries may have the highest potential as functional ingredients in food matrices.

2. Among all the tested infusions, the 96% ethanol infusion of rhubarb root showed the highest AB effect against all the tested bacteria including probiotic ones. Rhubarb can be highlighted as promising candidate for use as natural antibacterial in food. However, this integration cannot be recommended before proper health risk assessment of rhubarb root as a food component has been made.

3. Berries of the blue honeysuckle, bilberry, blackcurrant and sea buckthorn may have potential for use in the food industry as natural antioxidants and/or antimicrobials and functional ingredients in foods, as these berries did not supress the growth of probiotic bacteria and they additionally had good FRS properties.

4. During the analysis of FRS properties of the plant extracts, it was concluded that all the plant samples have to be diluted sufficiently to be in the linear range of the calibrant concentration.

5. Although in paper **III**, the tested plant extracts had a stronger AB effect than sodium nitrite solutions, none of the authors recommends substituting sodium nitrite in the processed meat products entirely with plant materials. Many more tests are needed before such recommendations can be made, especially taking into account hazards, related to bacterial species like *Clostridium botulinum* and other *Clostridia*.

6. A co-result of compiling different data for preparation of the present thesis is that no comprehensive database, containing complete nutritional data, especially on content of organic acids in the studied

cultivars, was found. The databases used: FINELI (2021), USDA Food Composition Database (2019) and NutriData (2021), contained some information, but not about all of the studied berries; data on blue honeysuckle was completely missing. A more complete database could give an opportunity to use the nutritional data in statistical analyses or for calculations of the daily menus.

7. In future studies it is necessary to determine, which polyphenolic or other compounds in a plant material have stronger FRS or AB properties. Furthermore, the plant materials have to be standardized for these compounds to achieve a similar effect in every case when these materials are used in foods.

8. In future studies, for example, the following aspects need to be focused on more systematically:

    a) To analyse statistically the differences between plant species in relation to their *in vitro* properties, by comparing them pairwise with more repetitions of each plant.

    b) To find the optimal extraction time to maintain the maximal AB and FRS properties of plant material.

    c) To analyse the effect of heating of the plant materials on their AB and FRS properties.

    d) To compare statistically the AB and FRS properties of the studied plants with some frequently used herbs or spices, like rosemary, oregano, garlic and others.

8. REFERENCES

Aaby, K., Grimmer, S., Holtung, L., 2013. Extraction of phenolic compounds from bilberry (*Vaccinium myrtillus* L.) press residue: Effects on phenolic composition and cell proliferation. LWT- Food Science and Technology, 54, 257–264.

Abdel-Massih, R.M., Abraham, A., 2014. Extracts of *Rosmarinus officinalis*, *Rheum rhaponticum*, and *Origanum majorana* exhibit significant anti-staphylococcal activity. International Journal of Pharmaceutical Sciences and Research, 5, 819–828.

Actis-Goretta, L., Ottaviani, J.I., Fraga, C.G., 2006. Inhibition of angiotensin converting enzyme activity by flavanol-rich foods. Journal of Agricultural and Food Chemistry, 54, 229–234.

Afanas'ev, I.B., Dcrozhko, A.I., Brodskii, A.V., Kostyuk, V.A., Potapovitch, A.I., 1989. Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. Biochemical Pharmacology, 38, 1763–1769.

Ahmad, W., Zaidi, S.M.A., Mujeeb, M., Ansari, S.H., Ahmad, S., 2014. HPLC and HPTLC methods by design for quantitative characterization and *in vitro* anti-oxidant activity of polyherbal formulation containing *Rheum emodi*. Journal of Chromatographic Science, 52, 911–918.

Aleixandre-Tudo, J.L., Buica, A. Nieuwoudt, H., Aleixandre, J.L., du Toit, W., 2017. Spectrophotometric analysis of phenolic compounds in grapes and wines. Journal of Agricultural and Food Chemistry, 65, 4009–4026.

Almeida, A.F., Borge, G.I.A., Piskula, M., Tudose, A., Tudoreanu, L., Valentová, K., Williamson, G., Santos, C.N., 2018. Bioavailability of quercetin in humans with a focus on interindividual variation. Comprehensive Reviews in Food Science and Food Safety, 17, 714–731.

Altunkaya, A., Gökmen, V., 2008. Effect of various inhibitors on enzymatic browning, antioxidant activity and total phenol content of fresh lettuce (*Lactuca sativa*). Food Chemistry, 107, 1173–1179.

Anton, D., Matt, D., Pedastsaar, P., Bender, I., Kazimierczak, R., Roasto, M., Kaart, T., Luik, A., Püssa, T., 2014. Three-year comparative study of polyphenol contents and antioxidant capacities in fruits of tomato (*Lycopersicon esculentum* Mill.) cultivars grown under organic and conventional conditions. Journal of Agricultural and Food Chemistry, 62, 5173–5180.

Anton, D., Bender, I., Kaart, T., Roasto, M., Heinonen, M., Luik, A., Püssa, T. 2017. Changes in polyphenols contents and antioxidant capacities of organically and conventionally cultivated tomato (*Solanum lycopersicum* L.) fruits during ripening. International Journal of Analytical Chemistry, 2017, 2367453.

Anton, D., Koskar, J., Raudsepp, P., Meremäe, K., Kaart, T., Püssa, T., Roasto, M., 2019. Antimicrobial and antioxidative effects of plant powders in raw and cooked minced pork. Foods, 8, 661.

Arus, L., Rätsep, R., Tammik M.-L., Zimmer, E., 2018. Väike kuslapuuraamat. Eesti Maaülikooli Polli Aiandusuuringute Keskus, 1–35.

Balouiri, M., Sadiki, M., Ibnsouda, S.K., 2016. Methods for *in vitro* evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis, 6, 71–79.

Bandonienė, D., Murkovic, M., 2002. On-line HPLC-DPPH screening method for evaluation of radical scavenging phenols extracted from apples (*Malus domestica* L.) Journal of Agricultural and Food Chemistry, 50, 2482–2487.

Basheer, L., Kerem, Z., 2015. Interactions between CYP3A4 and dietary polyphenols. Oxidative Medicine and Cellular Longevity, 2015, 854015.

Bisset, N.G., 1994. *Ribis nigri folium*, in: Herbal Drugs and Phytopharmaceuticals, CRC Press, London, pp. 421–423.

Bisset, N. G., Wichtl, M., 1994. Herbal drugs and phytopharmaceuticals: A handbook for practice on scientific basis. Medpharm Scientific Publishers.

Blainski, A., Lopes, G., de Mello, J., 2013. Application and analysis of the Folin-Ciocalteu method for the determination of the total phenolic content from *Limonium brasiliense* L. Molecules, 18, 6852–6865.

Boncler, M., Golanski, J., Lukasiak, M., Redzynia, M., Dastych, J., Watala, C., 2017. A new approach for the assessment of the toxicity of polyphenol-rich compounds with the use of high content screening analysis. PloS One, 12, 0180022.

British Growers Association, 2019. http://britishgrowers.org/british-growing/, viewed at 28.02.2019.

Bräunlich, M., Økstad, O., Slimestad, R., Wangensteen, H., Malterud, K., Barsett, H., 2013. Effects of *Aronia melanocarpa* constituents on biofilm formation of *Escherichia coli* and *Bacillus cereus*. Molecules, 18, 14989–14999.

Burri, S.C.M., Ekholm, A., Håkansson, Å., Tornberg, E., Rumpunen, K., 2017. Antioxidant capacity and major phenol compounds of horticultural plant materials not usually used. Journal of Functional Foods, 38, 119–127.

Caprioli, G., Iannarelli, R., Innocenti, M., Bellumori, M., Fiorini, D., Sagratini, G., Vittori, S., Buccioni, M., Santinelli, C., Bramucci, M., Quassinti, L., 2016. Blue honeysuckle fruit (*Lonicera caerulea* L.) from eastern Russia: phenolic composition, nutritional value and biological activities of its polar extracts. Food & Function, 7, 1892–1903.

Cardona, F., Andrés-Lacueva, C., Tulipani, S., Tinahones, F. J., Queipo-Ortuño, M.I., 2013. Benefits of polyphenols on gut microbiota and implications in human health. Journal of Nutritional Biochemistry, 24, 1415–1422.

Chai, Y.Y., Wang, F., Li, Y.L., Liu, K., Xu, H., 2012. Antioxidant activities of stilbenoids from *Rheum emodi* Wall. Evidence-based Complementary and Alternative Medicine, 2012, 603678.

Chaovanalikit, A., Thompson, M.M., Wrolstad, R.E., 2004. Characterization and quantification of anthocyanins and polyphenolics in blue honeysuckle (*Lonicera caerulea* L.). Journal of Agricultural and Food Chemistry, 52, 848–852.

CLSI, 2012. Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standard, 7th ed., CLSI document M02-A11. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA.

Colla, G., Kim, H-J., Kyriacou, M.C., Rouphael, Y., 2018. Nitrate in fruits and vegetables. Scientia Horticulturae, 237, 221–238.

Commission Regulation, 2012. (EU) No 432/2012; 02012R0432 — EN — 22.08.2017 — 009.001. https://eur-lex.europa.eu/eli/reg/2012/432, viewed 27.08.2021.

Coultate, T.P. 2009. FOOD. The Chemistry of its Components. 5th Edition. The Royal Society of Chemistry. Cambridge. UK., pp. 227–258.

Csepregi, K., Neugart, S., Schreiner, M., Hideg, É., 2016. Comparative evaluation of total antioxidant capacities of plant polyphenols. Molecules, 21, 208.

Česonienė, L., Labokas, J., Jasutienė, I., Šarkinas, A., Kaškonienė, V., Kaškonas, P., Kazernavičiūtė, R., Pažereckaitė, A., Daubaras, R., 2021. Bioactive compounds, antioxidant, and antibacterial properties of *Lonicera caerulea* berries: evaluation of 11 cultivars. Plants, 10, 624–638.

Daglia, M., 2012. Polyphenols as antimicrobial agents. Current Opinion in Biotechnology, 23, 174–181.

Day, A.J., Mellon, F., Barron, D., Sarrazin, G., Morgan, M.R., Williamson, G., 2001. Human metabolism of dietary flavonoids:

identification of plasma metabolites of quercetin. Free Radical Research, 35, 941–952.

Deffieux, D., Gaudrel-Grosay, S., Grelard, A., Chalumeau, C., Quideau, S., 2009. Stable solid-supported leucoanthocyanidin variants for flavanoid biosynthesis elucidation. Tetrahedron Letters, 50, 6567–6571.

de Rijke, E., Zappey, H., Ariese, F., Gooijer, C., Udo, A., 2003. Liquid chromatography with atmospheric pressure chemical ionization and electrospray ionization mass spectrometry of flavonoids with triple-quadrupole and ion-trap instruments. Journal of Chromatography A, 984, 45–58.

Declume, C., 1989. Anti-inflammatory evaluation of a hydroalcoholic extract of black currant leaves (*Ribes nigrum*). Journal of Ethnopharmacology, 27, 91–98.

Durazzo, A., Lucarini, M., 2019. Extractable and non-extractable antioxidants. Molecules, 24, 1933.

Dvaranauskaite, A., Venskutonis, P.R., Raynaud, C., Talou, T., Viškelis, P., Dambrauskiene, E., 2008. Characterization of steam volatiles in the essential oil of black currant buds and the antioxidant properties of different bud extracts. Journal of Agricultural and Food Chemistry, 56, 3279–3286.

ESCOP, 2003. E/S/C/O/P Monographs: *Ribis nigri folium*, London, pp. 425–428.

Eestikeelsete taimenimede andmebaas, 2021. http://taimenimed.ut.ee/, viewed 27.01.2021.

EFSA, 2011. Panel on Dietetic Products. Nutrition and allergies (NDA). Scientific opinion on the substantiation of health claims related to polyphenols in olive oil and protection of LDL particles from oxidative damage (ID 1333, 1638, 1639, 1696, 2865), maintenance of normal blood HDL-cholesterol concentrations (ID 1639), maintenance of normal blood pressure (ID 3781), "anti-inflammatory properties" (ID 1882), "contributes to the upper

respiratory tract health" (ID 3468), "can help to maintain a normal function of gastrointestinal tract" (3779), and "contributes to body defences against external agents" (ID 3467) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal, 9, 2033, viewed 27.08.2021.

Emiliani, G., Fondi, M., Fani, R., Gribaldo, S., 2009. A horizontal gene transfer at the origin of phenylpropanoid metabolism: a key adaptation of plants to land. Biology Direct, 4, 7.

Eriksson, C.E., 1982. Lipid oxidation catalysts and inhibitors in raw materials and processed foods. Food Chemistry, 9, 3–19.

EU-NOMEN, 2021. Period 2008-2011, http://www.eu-nomen.eu/portal/taxon.php?GUID=F8CA964E-1D4E-4C57-8C9B-1CB7A554256D#, viewed 21.04.2021.

European Pharmacopoeia (Ph. Eur.), 9th Edition, 2019. https://www.edqm.eu/en/european-pharmacopoeia-ph-eur-9th-edition, viewed 08.02.2019.

FINELI, 2021. https://fineli.fi/fineli/fi/elintarvikkeet/442, viewed 27. 01. 2021.

Flora of North America, 2005. Flora of North America: North of Mexico Volume 5: Magnoliophyta: Caryophyllidae, part 2. http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=242343402, viewed 28.01.2021.

Fraga, C.G., Croft, K.D., Kennedy, D.O., Tomás-Barberán, F.A., 2019. The effects of polyphenols and other bioactives on human health. Food & Function, 10, 514–528.

Garbacki, N., Kinet, M., Nusgens, B., Desmecht D., Damas, J., 2005. Proanthocyanidins, from *Ribes nigrum* leaves, reduce endothelial adhesion molecules ICAM-1 and VCAM-1. Journal of Inflammation, 2, 9.

Girones-Vilaplana, A., Valentao, P., Andrade, P.B., Ferreres, F., Moreno, D.A., Garcia-Viguera, C., 2012. Phytochemical profile of a

blend of black chokeberry and lemon juice with cholinesterase inhibitory effect and antioxidant potential. Food Chemistry, 134, 2090–2096.

Goni, P., López, P., Sánchez, C., Gómez-Lus, R., Becerril, R., Nerín, C., 2009. Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. Food Chemistry, 116, 982–989.

Gómez-Romero, M., Arráez-Román, D., Segura-Carretero, A., Fernández-Gutiérrez, A., 2007. Analytical determination of antioxidants in tomato: typical components of the Mediterranean diet. Journal of Separation Science, 30, 452–461.

Gómez-Romero, M., Segura-Carretero, A., Fernjndez-Gutiérrez, A., 2010. Metabolite profiling and quantification of phenolic compounds in methanol extracts of tomato fruit. Phytochemistry, 71, 1848–1864.

Grayer R.J., Kokubun, T., 2001. Plant-fungal interactions: the search for phytoalexins and other antifungal compounds from higher plants. Phytochemistry, 56, 253–263.

Grosso, G., 2018. Effects of polyphenol-rich foods on human health. Nutrients, 10, 1089–1096.

Hardman, W.E., 2014. Diet components can suppress inflammation and reduce cancer risk. Nutrition Research and Practice, 8, 233–240.

Harris, C.S., Burt, A.J., Saleem, A. Le, P.M., Martineau, L.C., Haddad, P.S., Benett, S.A.L., Arnason, J.T., 2007. A single HPLC-PAD-APCI/MS method for quantitative comparison of phenolic compounds found in leaf, stem, root and fruit extracts of *Vaccinium angustifolium*. Phytochemical Analysis, 18, 161–169.

Hasper, I., Ventskovskiy, B.M., Rettenberger, R., Heger, P.W., Riley, D.S., Kaszkin-Bettag, M., 2009. Long-term efficacy and safety of the special extract ERr 731 of *Rheum rhaponticum* in perimenopausal women with menopausal symptoms. Menopause, 16, 117–131.

He, J., Magnuson, B.A., Lala, G., Tian, Q., Schwartz, S.J., Giusti, M.M., 2006. Intact anthocyanins and metabolites in rat urine and plasma

after 3 months of anthocyanin supplementation. Nutrition and Cancer, 54, 3–12.

Heger, M, Ventskovskiy, B.M., Borzenko, I., Kneis, K.C., Rettenberger, R., Kaszkin-Bettag M., Heger, P.W., 2006. Efficacy and safety of a special extract of *Rheum rhaponticum* (ERr 731) in perimenopausal women with climacteric complaints: a 12-week randomized, double-blind, placebo-controlled trial. Menopause, 13, 744–759.

Heinonen, M., 2007. Antioxidant activity and antimicrobial effect of berry phenolics–a Finnish perspective. Molecular Nutrition & Food Research, 51, 684–691.

Helmja, K., Vaher, M., Püssa, T., Raudsepp, P., Kaljurand, M., 2008. Evaluation of antioxidative capability of the tomato (*Solanum lycopersicum*) skin constituents by capillary electrophoresis and high-performance liquid chromatography. Electrophoresis, 29, 3980–3988.

Hmelak Gorenjak, A., Cencič, A., 2013. Nitrate in vegetables and their impact on human health. A review. Acta Alimentaria, 42, 158–172.

Hohmann, C.D., Cramer, H., Michalsen, A., Kessler, C., Steckhan, N., Choi, K., Dobos, G., 2015. Effects of high phenolic olive oil on cardiovascular risk factors: A systematic review and meta-analysis. Phytomedicine, 22, 631–640.

Huang, D., Ou, B., Prior, R., 2005. The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry, 53, 1841–1856.

IBA, 2017. Global blackcurrant production, https://www.blackcurrant-iba.com/wp-content/uploads/2018/06/Global-BC-Production-2009-2017.pdf, viewed at 28.01.2021.

Ieri, F., Innocentib, M., Possieric, L., Gallorib, S., Mulinacci, N., 2015. Phenolic composition of "bud extracts" of *Ribes nigrum* L., *Rosa canina* L. and *Tilia tomentosa* M. Journal of Pharmaceutical and Biomedical Analysis, 115, 1–9.

Iizuka, A., Iijima, O.T., Kondo, K., Itakura, H., Yoshie, F., Miyamoto, H., Kubo, M., Higuchi, M., Takeda, H., Matsumiya, T., 2004. Evaluation of Rhubarb using antioxidative activity as an index of pharmacological usefulness. Journal of Ethnopharmacology, 91, 89–94.

Jakobek, L., Šeruga, M., Novak, I. and Medvidović-Kosanović, M., 2007. Flavonols, phenolic acids and antioxidant activity of some red fruits. Deutsche Lebensmittel-Rundschau, 103, 369–378.

Jalakas, M., Kelt, K., Karp, K., 2003. The yield and fruit quality of sea buckthorn (*Hippophae rhamnoides* L.) after rejuvenation cutting. Agronomy Research, 1, 31–36.

Jeandet, P., Vannozzi, A., Sobarzo-Sánchez, E., Uddin, M.S., Bru, R., Martínez-Márquez, A., Clément, C., Cordelier, S., Manayi, A., Nabavi, S.F., Rasekhian, M., 2021. Phytostilbenes as agrochemicals: biosynthesis, bioactivity, metabolic engineering and biotechnology. Natural Product Reports, 38, 1282-1329.

Jurikova, T., Sochor, J., Rop, O., Mlček, J., Balla, Š., Szekeres, L., Žitný, R., Zitka, O., Adam, V., Kizek, R., 2012. Evaluation of polyphenolic profile and nutritional value of non-traditional fruit species in the Czech Republic — a comparative study. Molecules, 17, 8968–8981.

Kapp, K., Hakala, E., Orav, A., Pohjala, L., Vuorela, P., Püssa, T., Vuorela, H., Raal, A., 2013. Commercial peppermint (*Mentha×piperita* L.) teas: Antichlamydial effect and polyphenolic composition. Food Research International, 53, 758–766.

Kalisz, S., Oszmiański, J., Kolniak-Ostek, J., Grobelna, A., Kieliszek, M., Cendrowski, A., 2020. Effect of a variety of polyphenols compounds and antioxidant properties of rhubarb (*Rheum rhabarbarum*). LWT-Food Science and Technology, 118, 108775.

Kalliala, H., Kauste, O., 1964. Ingestion of rhubarb leaves as cause of oxalic acid poisoning. Annales Paediatrae Fenniae, 10, 228–231.

Kikas, A., Libek, A.-V., Kelt, K., Raudsepp, P., Kahu, K., Vahejõe, K., Pennar, M., 2008. Musta sõstra kasvatus. Eesti Maaülikool.

Kimble, R., Keane, K.M., Lodge, J.K., Howatson, G., 2018. Dietary intake of anthocyanins and risk of cardiovascular disease: A systematic review and meta-analysis of prospective cohort studies. Critical Reviews in Food Science and Nutrition, 59, 3032–3043.

Kong, J.M., Chia, L.S., Goh, N.K., Chia, T.F., Brouillard, R., 2003. Analysis and biological activities of anthocyanins. Phytochemistry, 64, 923–933.

Kolodziejczyk-Czepas, J., Liudvytska, O., 2020. *Rheum rhaponticum* and *Rheum rhabarbarum*: A review of phytochemistry, biological activities and therapeutic potential. Phytochemistry Reviews, 20, 589–607.

Komatsu, K., Nagayama, Y., Tanaka, K., Ling, Y., Basnet, P., Meselhy, M.R., 2006. Development of a high performance liquid chromatographic method for systematic quantitative analysis of chemical constituents in rhubarb. Chemical and Pharmaceutical Bulletin, 54, 941–947.

Koskar, J., Kramarenko, T., Meremäe, K., Kuningas, M., Sõgel, J., Mäesaar, M., Anton, D., Lillenberg, M., Roasto, M., 2019. Prevalence and numbers of *Listeria monocytogenes* in various ready-to-eat foods over a 5-year period in Estonia. Journal of Food Protection, 82, 597–604.

Kosikowska, U., Smolarz, H., Malm, A., 2010. Antimicrobial activity and total content of polyphenols of *Rheum* L. species growing in Poland. Open Life Sciences, 5, 814–820.

Kylli, P., 2011. Berry phenolics: isolation, analysis, identification, and antioxidant properties. ACADEMIC DISSERTATION. ISBN: 978-952-10-7115-7. University of Helsinki, Department of Food and Environmental Sciences, Food Chemistry. Unigrafia, Helsinki.

Lapornik, B., Prošek, M., Wondra, A.G., 2005. Comparison of extracts prepared from plant by-products using different solvents and extraction time. Journal of Food Engineering, 71, 214–222.

Lee, J., Durst, R., Wrolstad, R.E., 2006. Total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines- pH differential method. Journal of AOAC International, 88, 1269–1278.

Lee, J.E., Kim, G.S., Park, S., Kim, Y.H., Kim, M.B., Lee, W.S., Jeong, S.W., Lee, S.J., Jin, J.S., Shin, S.C., 2014. Determination of chokeberry (*Aronia melanocarpa*) polyphenol components using liquid chromatography–tandem mass spectrometry: Overall contribution to antioxidant activity. Food Chemistry, 146, 1–5.

Li, L., Song, X., Yin, Z., Jia, R., Li, Z., Zhou, X., Zou, Y., Li, L., Yin, L., Yue, G., Ye, G., 2016. The antibacterial activity and action mechanism of emodin from *Polygonum cuspidatum* against *Haemophilus parasuis in vitro*. Microbiological Research, 186, 139–145.

Libek, A., Kikas, A., Kaldmäe, H., Arus, L., 2008. Blackcurrant breeding in Estonia. Acta Horticulturae, 777, 77–80.

Libek, A.-V., Kahu, K., Volens, K., Kikas, A., 2017. Mahepõllumajandusse sobivate marja- ja puuviljade sordikataloog. Eesti Maaülikool.

Lu, C., Wang, H., Lv, W., Xu, P., Zhu, J., Xie, J., Liu, B., Lou, Z., 2011. Antibacterial properties of anthraquinones extracted from rhubarb against *Aeromonas hydrophila*. Fisheries Science, 77, 375.

Maaeluministeerium, 2018. Põllumajanduse, kalanduse ja toiduainetööstuse ülevaade, https://www.agri.ee/sites/default/files/content/ylevaated/ulevaade-pokat-2018.pdf, viewed 28.01.2021

Ma, D.S., Tan, L.T.H., Chan, K.G., Yap, W.H., Pusparajah, P., Chuah, L.H., Ming, L.C., Khan, T.M., Lee, L.H., Goh, B.H., 2018. Resveratrol—potential antibacterial agent against foodborne pathogens. Frontiers in Pharmacology, 9, 102–118.

Manach, C., Williamson, G., Morand, C., Scalbert, A., Rémésy, C., 2005. Bioavailability and bioefficacy of polyphenols in humans. I. Review

of 97 bioavailability studies. The American Journal of Clinical Nutrition, 81, 230S–242S.

Manou, I., Bouillard, L., Devleeschouwer, M.J. Barel, A.O., 1998. Evaluation of the preservative properties of *Thymus vulgaris* essential oil in topically applied formulations under a challenge test. Journal of Applied Microbiology, 84, 368–376.

Marconi, O., Floridi, S., Montanari, L., 2007. Organic acids profile in tomato juice by HPLC with UV detection. Journal of Food Quality, 30, 253–266.

Mattila, P.H., Hellström, J., Karhu, S., Pihlava, J.M., Veteläinen, M., 2016. High variability in flavonoid contents and composition between different North-European currant (*Ribes* spp.) varieties. Food Chemistry, 204, 14–20.

Mennen, L.I., Walker, R., Bennetau-Pelissero, C., Scalbert, A., 2005. Risks and safety of polyphenol consumption. The American Journal of Clinical Nutrition, 81, 326S–329S.

Mikulic-Petkovsek, M., Schmitzer, V., Slatnar, A., Stampar, F., Veberic, R., 2012. Composition of sugars, organic acids, and total phenolics in 25 wild or cultivated berry species. Journal of Food Science, 77, 1064–1070.

Milivojević, J., Bogdanović-Pristov, J., Maksimović, V., 2010. Phenolic compounds and vitamin C as sources of antioxidant activity in black currant fruit (*Ribes nigrum* L.). Acta Agriculturae Serbica, 15, 3–10.

Miura, T., Muraoka, S., Ikeda, N., Watanabe, M., Fujimoto, Y., 2000. Antioxidative and prooxidative action of stilbene derivatives. Pharmacology & Toxicology, 86, 203–208.

Nadõrova. D., 2007. Polüfenoolide ja tokoferoolide võrdlev fütokeemiline analüüs hariliku astelpaju (*Hippophae rhamnoides* L.) sortides. Proviisoriõppe lõputöö. Tartu Ülikool, Farmaatsia Instituut, Tartu 2007.

Nepomuceno, J.C., 2011. Antioxidants in cancer treatment. In "Current Cancer Treatment – Novel Beyond Conventional Approaches". Chapter 27, pp. 623–650.

Noh, H., Freisling, H., Assi, N., Zamora-Ros, R., Achaintre, D., Affret, A., Mancini, F., Boutron-Ruault, M.C., Flögel, A., Boeing, H., Kühn, T., Schübel, R., Trichopoulou, A., Naska, A., Kritikou, M., Palli, D., Pala, V., Tumino, R., Ricceri, F., Santucci de Magistris, M., Cross, A., Slimani, N., Scalbert, A., Ferrari, P., 2017. Identification of urinary polyphenol metabolite patterns associated with polyphenol-rich food intake in adults from four European countries. Nutrients, 9, 796.

Nour, V., Stampar, F., Veberic, R., Jakopic, J., 2013. Anthocyanins profile, total phenolics and antioxidant activity of black currant ethanolic extracts as influenced by genotype and ethanol concentration. Food Chemistry, 141, 961–966.

Nowak, A., Czyzowska, A., Efenberger, M., Krala, L., 2016. Polyphenolic extracts of cherry (*Prunus cerasus* L.) and blackcurrant (*Ribes nigrum* L.) leaves as natural preservatives in meat products. Food Microbiology, 59, 142–149.

NuitriData, 2019. ©NutriData food composition database, version 9.0, tka.nutridata.ee, 2019. https://tka.nutridata.ee/en/, viewed 24. 02. 2021.

Oszmiański, J., Wojdylo, A., 2005. *Aronia melanocarpa* phenolics and their antioxidant activity. European Food Research and Technology, 221, 809–813.

Papastergiadis, A., Mubiru, E., Van Langenhove, H., De Meulenaer, B., 2012. Malondialdehyde measurement in oxidized foods: evaluation of the spectrophotometric thiobarbituric acid reactive substances (TBARS) test in various foods. Journal of Agricultural and Food Chemistry, 60, 9589–9594.

Papuc, C., Goran, G.V., Predescu, C.N., Nicorescu, V., Stefan, G., 2017. Plant polyphenols as antioxidant and antibacterial agents for shelf-life extension of meat and meat products: classification, structures,

sources, and action mechanisms. Comprehensive Reviews in Food Science and Food Safety, 16, 1243–1268.

Paśko, P., Bartoń, H., Zagrodzki, P., Gorinstein, S., Fołta, M., Zachwieja, Z., 2009. Anthocyanins, total polyphenols and antioxidant activity in amaranth and quinoa seeds and sprouts during their growth. Food Chemistry, 115, 994–998.

Pepkolaj, A., Faslia, N., Ozuni, E., 2017. Chemical variability of wild bilberry (*Vaccinium myrtillus* L.) populations in northern part of Albania. Journal of Medicinal Plants Research, 11, 576–582.

Perveen, R., Suleria, H.A.R., Anjum, F.M., Butt, M.S., Pasha, I., Ahmad, S., 2015. Tomato (*Solanum lycopersicum*) carotenoids and lycopenes chemistry; metabolism, absorption, nutrition, and allied health claims—a comprehensive review. Critical Reviews in Food Science and Nutrition, 55, 919–929.

Piwowarski, J.P., Granica, S., Stefańska, J., Kiss, A.K., 2016. Differences in metabolism of ellagitannins by human gut microbiota *ex vivo* cultures. Journal of Natural Products, 79, 3022–3030.

Pop, R.M., Socaciu, C., Pintea, A., Buzoianu, A.D., Sanders, M.G., Gruppen, H., Vincken, J.P., 2013. UHPLC/PDA–ESI/MS analysis of the main berry and leaf flavonol glycosides from different Carpathian *Hippophaë rhamnoides* L. varieties. Phytochemical Analysis, 24, 484–492.

Püssa, T., Raudsepp, P., Toomik, P., Pällin, R., Mäeorg, U., Kuusik, S., Soidla, R., Rei, M., 2009. A study of oxidation products of free polyunsaturated fatty acids in mechanically deboned meat. Journal of Food Composition and Analysis, 22, 307–314.

Quideau, S., Deffieux, D., Douat-Casassus, C., Pouysegu, L., 2011. Plant polyphenols: chemical properties, biological activities, and synthesis. Angewandte Chemie International Edition, 50, 586–621.

Raudsepp, P., Libek, E., Rätsep, R., Kikas, A., Libek, A.-V., Arus, L., Kaldmäe, H., Bleive, U., 2017. Antotsüaanide sisalduse varieeruvus ja

säilimine mõnedes Eestimaal kasvavates marjades. Terve loom ja tervislik toit, 61–66. Eesti Maaülikool.

Renard, C.M.G.C., 2017. Bioactives in fruit and vegetables and their extraction: state of the art and perspectives. Conference presentation: Bio2Actives 2017. Quimper, France.

Renard, C.M.G.C., 2018. Extraction of bioactives from fruit and vegetables: State of the art and perspectives. LWT- Food Science and Technology, 93, 390–395.

Reuveni, R., Reuveni, M., 1998. Foliar-fertilizer therapy—a concept in integrated pest management. Crop Protection, 17, 111–118.

Rumpunen, K., Henriksen, K., 1999. Phytochemical and morphological characterization of seventy-one cultivars and selections of culinary rhubarb (*Rheum* spp.). The Journal of Horticultural Science and Biotechnology, 74, 13–18.

Rupasinghe, H.P.V., Arumuggam, N., Amararathna, M., De Silva, A.B.K.H., 2018. The potential health benefits of haskap (*Lonicera caerulea* L.): Role of cyanidin-3-O-glucoside. Journal of Functional Foods, 44, 24–39.

Rusak, G., Komes, D., Likić, S., Horžić, D., Kovač, M., 2008. Phenolic content and antioxidative capacity of green and white tea extracts depending on extraction conditions and the solvent used, Food Chemistry, 110, 852–858.

Sarneckis, C.J., Dambergs, R.G., Jones, P., Mercurio, M., Herderich, M.J., Smith, P.A., 2006. Quantification of condensed tannins by precipitation with methyl cellulose: development and validation of an optimised tool for grape and wine analysis. Australian Journal of Grape and Wine Research, 12, 39–49.

Scalzo, J., Currie, A., Stephens, J., McGhie, T., Alspach, P., 2008. The anthocyanin composition of different *Vaccinium*, *Ribes* and *Rubus* genotypes. BioFactors, 34, 13–21.

Sebiomo, A., Awofodu, A.D., Awosanya, A.O., Awotona, F.E., Ajayi, A.J., 2011. Comparative studies of antibacterial effect of some antibiotics and ginger (*Zingiber officinale*) on two pathogenic bacteria. Journal of Microbiology and Antimicrobials, 3, 18–22.

Sergediene, E., Jönsson, K., Szymusiak, H., Tyrakowska, B., Rietjens, I.M., Narimantas, Č., 1999. Prooxidant toxicity of polyphenolic antioxidants to HL-60 cells: description of quantitative structure-activity relationships. FEBS Letters, 462, 392–396.

Shih, P.H., Yeh, C.T., Yen G.C., 2007. Anthocyanins induce the activation of phase II enzymes through the antioxidant response element pathway against oxidative stress-induced apoptosis. Journal of Agricultural and Food Chemistry, 55, 9427–9435.

Shutterstock, 2021. https://teachmephysiology.com/immune-system/infections/pathogens/, viewed 24. 02. 2021

Slimestad, R., Torskangerpoll, K., Nateland, H. S., Johannessen, T., Giske, N. H., 2005. Flavonoids from black chokeberries, *Aronia melanocarpa*. Journal of Food Composition and Analysis, 18, 61–68.

Sordivaramu, 2021. Eesti Maaülikool. http://sordivaramu.emu.ee/sort2. php?id=52, viewed 28. 01. 2021

Stević, T., Šavikin, K., Ristić, M., Zdunić, G., Janković, T., Krivokuća-Đokić, D., Vulić, T., 2010. Composition and antimicrobial activity of the essential oil of the leaves of black currant (*Ribes nigrum* L.) cultivar Čačanska crna. Journal of the Serbian Chemical Society, 75, 35–43.

Steyn, W.J., Wand, S.J.E., Holcroft, D.M., Jacobs, G., 2002. Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. New Phytologist, 155, 349–361.

SUSMEATPRO, 2021. https://sites.google.com/site/susmeatpro/home, viewed 28. 01. 2021.

Šenica, M., Bavec M., Stampar F., Mikulic-Petkovsek, M., 2018. Blue honeysuckle (*Lonicera caerulea subsp. edulis* (Turcz. Ex Herder) Hultén.)

berries and changes in their ingredients across different locations. Journal of the Science of Food and Agriculture, 98, 3333–3342.

Tabart, J., Kevers, C., Pincemail, J., Defraigne, J.O., Dommes, J., 2006. Antioxidant capacity of black currant varies with organ, season, and cultivar. Journal of Agricultural and Food Chemistry, 54, 6271–6276.

Taguchi, C., Fukushima Y., Kishimoto Y., Suzuki-Sugihara N., Saita, E., Takahashi, Y., Kondo, K., 2015. Estimated dietary polyphenol intake and major food and beverage sources among elderly Japanese. Nutrients, 7, 10269–10281.

Taguri, T., Tanaka, T., Kouno, I., 2006. Antibacterial spectrum of plant polyphenols and extracts depending upon hydroxyphenyl structure. Biological and Pharmaceutical Bulletin, 29, 2226–2235.

Takeoka, G.R., Dao, L. Harden, L. Pantoja, A., Kuhl, J.C., 2013. Antioxidant activity, phenolic and anthocyanin contents of various rhubarb (*Rheum* spp.) varieties. International Journal of Food Science and Technology, 48, 172–178.

Tian, L., Pang, Y., Dixon, R.A., 2008. Biosynthesis and genetic engineering of proanthocyanidins and (iso) flavonoids. Phytochemistry Reviews, 7, 445–465.

Tian, Y., Liimatainen, J., Alanne, A.-L., Lindstedt, A., Liu, P., Sinkkonen, J., Kallio, H., Yang, B., 2017. Phenolic compounds extracted by acidic aqueous ethanol from berries and leaves of different berry plants. Food Chemistry, 220, 266–281.

Tian, Y., Puganen, A., Alakomi, H.L., Uusitupa, A., Saarela, M., Yang, B., 2018. Antioxidative and antibacterial activities of aqueous ethanol extracts of berries, leaves, and branches of berry plants. Food Research International, 106, 291–303.

Tian, Y., Laaksonen, O.A., Haikonen, H., Vanag, A., Ejaz, H., Linderborg, K.M., Karhu, S., Yang, B., 2019. Compositional diversity among blackcurrant (*Ribes nigrum*) cultivars originating from European countries. Journal of Agricultural and Food Chemistry, 67, 5621−5633.

Tomás-Barberán, F.A., Espín, J.C., 2019. Effect of food structure and processing on (poly)phenol–gut microbiota interactions and the effects on human health. Annual Review of Food Science and Technology, 10, 221–238.

Tonin, F., Steimbach, L., Wiens, A., Perlin, C., Pontarolo, R., 2015. Impact of natural juice consumption on plasma antioxidant status: a systematic review and meta-analysis. Molecules, 20, 22146–22156.

USDA, 2019. https://ndb.nal.usda.gov/ndb/search/list, viewed 28. 01. 2021.

Vagiri, M., Ekholm, A., Andersson, S.C., Johansson, E., Rumpunen, K., 2012. An optimized method for analysis of phenolic compounds in buds, leaves, and fruits of black currant (*Ribes nigrum* L.). Journal of Agricultural and Food Chemistry, 60, 10501–10510.

Vagiri, M., Ekholm, A., Oberg, E., Johansson, E., Andersson, S.C., Rumpunen, K., 2013. Phenols and ascorbic acid in black currant (*Ribes nigrum* L.): variation due to genotype, location and year. Journal of Agricultural and Food Chemistry, 61, 9298–9306.

Vagiri, M.R., 2014. Phenolic compounds and ascorbic acid in black currant (*Ribes nigrum* L.) variation due to genotype, ontogenetic stage, harvest date and location. DOCTORAL THESIS. ISBN: 978-91-576-8135-5. Swedish University of Agricultural Sciences, Faculty of Landscape Architecture, Horticulture and Crop Production Science, Department of Plant Breeding. Acta Universitatis Agriculturae Sueciae, Alnarp.

Vagiri, M., Conner, S., Stewart, D., Andersson, S.C., Verrall, S., Johansson, E., Rumpunen, K., 2015. Phenolic compounds in blackcurrant (*Ribes nigrum* L.) leaves relative to leaf position and harvest date. Food Chemistry, 172, 135–142.

Vander Kloet, S.P., 1988. The genus Vaccinium in North America (No. 1828). Agriculture Canada.

Vankar, P.S., Srivastava, J., 2010. Evaluation of anthocyanin content in red and blue flowers. International Journal of Food Engineering, 6, 7.

Viljanen, K., Kylli, P., Hubbermann, E.M., Schwarz, K., Heinonen, M., 2005. Anthocyanin antioxidant activity and partition behavior in whey protein emulsion. Journal of Agricultural and Food Chemistry, 53, 2022–2027.

Villano, D., Fernández-Pachón, M.S., Moyá, M.L., Troncoso, A.M., García-Parrilla, M.C., 2007. Radical scavenging ability of polyphenolic compounds towards DPPH free radical. Talanta, 71, 230–235.

Wang, S., Hu, T., Zhang, F., Forrer, H.R., Cao, K., 2007. Screening for plant extracts to control potato late blight. Frontiers of Agriculture in China, 1, 43–46.

Wang, J., Zhao, H., Kong, W., Jin, C., Zhao, Y., Qu, Y., Xiao, X., 2010. Microcalorimetric assay on the antimicrobial property of five hydroxyanthraquinone derivatives in rhubarb (*Rheum palmatum* L.) to *Bifidobacterium adolescentis*. Phytomedicine, 17, 684–689.

Wang, X., Ouyang, Y.Y., Liu J., Zhao, G., 2014. Flavonoid intake and risk of CVD: a systematic review and meta-analysis of prospective cohort studies. British Journal of Nutrition, 111, 1–11.

Wangensteen, H., Bräunlich, M., Nikolic, V., Malterud, K.E., Slimestad, R., Barsett, H., 2014. Anthocyanins, proanthocyanidins and total phenolics in four cultivars of aronia: Antioxidant and enzyme inhibitory effects. Journal of Functional Foods 7, 746–752.

Wilhelm, M.J., Sheffield, J.B., Sharifian, Gh.M., Wu, Y., Spahr, C., Gonella, G., Xu, B., Dai, H.L., 2015. Gram's stain does not cross the bacterial cytoplasmic membrane. ACS Chemical Biology, 10, 1711–1717.

Winkel-Shirley, B., 2002. Biosynthesis of flavonoids and effects of stress. Current Opinion in Plant Biology, 5, 218–223.

Witzell, J., Gref, R., Näsholm, T., 2003. Plant-part specific and temporal variation in phenolic compounds of boreal bilberry (*Vaccinium myrtillus*) plants. Biochemical Systematics and Ecology 31, 115–127.

Xi, Y., Sullivan, G.A., Jackson, A.L., Zhou, G.H., Sebranek, J.G., 2011. Use of natural antimicrobials to improve the control of *Listeria monocytogenes* in a cured cooked meat model system. Meat Science, 88, 503–511.

Yen G.-C., Duh P.-D., Chuang D.-Y., 2000. Antioxidant activity of anthraquinones and anthrone. Food Chemistry 70, 437–441.

Yen, G.C., Duh, P.D., Tsai, H.L., 2002. Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid. Food Chemistry, 79, 307–313.

Zafra-Stone, S., Yasmin, T., Bagchi, M., Chatterjee, A., Vinson, J.A., Bagchi, D., 2007. Berry anthocyanins as novel antioxidants in human health and disease prevention. Molecular Nutrition & Food Research, 51, 675–683.

Zhang, D., Hamauzu, Y., 2004. Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. Food Chemistry, 88, 503–509.

Zhao, H., Wang, Z., Ma, F., Yang, X., Cheng, C., Yao, L., 2012. Protective effect of anthocyanin from *Lonicera caerulea var. edulis* on radiation-induced damage in mice. International Journal of Molecular Sciences, 13, 11773–11782.

Zheng, J., Yang, B., Ruusunen, V., Laaksonen, O., Tahvonen, R., Hellsten, J., Kallio, H., 2012. Compositional differences of phenolic compounds between black currant (*Ribes nigrum* L.) cultivars and their response to latitude and weather conditions. Journal of Agricultural and Food Chemistry 60, 6581–6593.

Ziad, D., Elias, A., Roula, A.M., 2011. Antibacterial activity of *Rheum rhaponticum*, *Olea europaea*, and *Viola odorata* on ESBL producing clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*. International Journal of Pharmaceutical Sciences and Research, 2, 1669–1678.

9. SUMMARY IN ESTONIAN

Hariliku rabarberi (*Rheum rhaponticum* L.) ja musta sõstra (*Ribes nigrum* L.) polüfenoolne koostis, nende taimede antibakteriaalse toime ja vabade radikaalide sidumise võime võrdlus mõnede teiste toidutaimedega

Sissejuhatus

Nii must sõstar (*Ribes nigrum* L.) kui ka harilik rabarber (*Rheum rhaponticum* L.) on maailmas tähtsad toidutaimed. Aastal 2017 kasvatati rahvusvahelise mustsõstrakasvatajate liidu IBA („International Black-currant Association", 2017) andmetel maailmas musta sõstart 55 616 hektaril (49 416 tonni), sealjuures Eestis 2018. aasta andmetel 626 hektaril 479 tonni aastas (Maaeluministeerium, 2018). Rabarberi-kasvatuse mahtude kohta Eestis andmed puuduvad, ent Briti kasvatajate ühingu andmetel kasvatati Suurbritannias 2019. aastal 25 000 tonni rabarberit („British Growers Association", 2019). Harilikul rabarberil ja mustal sõstral on peale toiteväärtuse leitud ka tervist tugevdavaid toimeid (Hasper jt, 2009; European Pharmacopoeia, 2019). Mõlemal taimeliigil on leitud nii antibakteriaalset (AB) toimet (Kosikowska jt, 2010; Ziad jt, 2011; Abdel-Massih ja Abraham, 2014; Nowak jt, 2016) kui ka vabade radikaalide sidumise (VRS) võimet, mida käsitletakse töös ka antioksüdantse (AO) toimena (Tabart jt, 2006; Jakobek jt, 2007; Milivojević jt, 2010; Chai jt, 2012; Ahmad jt, 2014).

Lisaks eelmainitud taimede polüfenoolse koostise uurimisele võrreldi käesolevas töös nende taimede eri osade (musta sõstra marjade ja lehtede ning hariliku rabarberi lehevarte ja juurte) VRS-i võimet ja AB toimet kahes erinevas eksperimendis *in vitro* tingimustes mõne teise toidutaime toimetega. Võrdluses kasutati hariliku mustika (*Vaccinium myrtillus* L.), söödava kuslapuu (*Lonicera caerulea* L.), aroonia (*Aronia melanocarpa* (Michx.) Elliott), astelpaju (*Hippophae rhamnoides* L.) ning tomati (*Lycopersicon esculentum* Mill.) vilju.

Töö käigus kogutud teadmisi kasutati ja arendati edasi rahvusvahelise projekti „Sustainable plant ingredients for healthier meat products – proof of concepts" (SUSMEATPRO) raames. Selle projekti eesmärk oli leida toidutaimi ja/või nende töötlemise käigus tekkivaid kõrvalsaadusi,

millel on antimikroobsed ja/või antioksüdantsed omadused ning mida saaks kasutada lihatoodetes antimikroobsete ja antioksüdantsete lisanditena lihatoodete tervislikumaks muutmisel. Käesolevas töös kajastatud artiklites avaldatud tulemusi kasutati selleks, et valida projekti järgmises etapis välja taimed, mida kasutati lihatoodetes antimikroobse ja antioksüdantse toime tugevdamiseks (Anton jt, 2019). Edasistes uurimistöödes jätkuvad erinevate taimsete materjalide AB toime ja VRS-i võime uuringud toidumaatriksites.

Käesoleva väitekirja eesmärgid

Töö peamine eesmärk oli leida mõnede eelnevalt välja valitud taimede hulgast selline/sellised, mis on nii tugevate vaba radikaali sidumise (VRS) võime kui ka antibakteriaalsete (AB) omadustega.

Selle eesmärgi täitmiseks oli vaja läbida järgnevad etapid:

1) määrata hariliku rabarberi lehevarte ja juurte polüfenoolne koostis (Artiklid **I** ja **IV**).

2) määrata musta sõstra marjade ja lehtede polüfenoolne koostis (Artiklid **II** ja **IV**).

3) võrrelda omavahel erinevate taimede toiduks sobivate ekstraktide VRS-i võimet ja AB toimet:

    a) võrrelda rabarberi lehevarsi ja juuri, söödava kuslapuu, tomati, hariliku mustika, astelpaju ja musta sõstra vilju (Artikkel **III**).

    b) võrrelda rabarberi lehevarsi ja juuri, musta sõstra lehti ja vilju ning aroonia ja söödava kuslapuu vilju (Artikkel **IV**).

Kasutatud materjalid ja meetodid

Artiklis **I** kasutati rabarberi juure ja lehevarte polüfenoolse koostise uurimiseks alljärgneval viisil kogutud materjale: Põhja-Eestist, Kuusalu lähistelt koguti hariliku rabarberi taimelt 50 g kaupa proove. Näidiseksemplarid säilitati Tartu Ülikooli Farmaatsiainstituudis (Nooruse 1, Tartu 50411, Eesti, proov nr. 715). Proovid lõigati umbes 1x5 cm tükkideks ja kuivatati 10 päeva toatemperatuuril, hästi ventileeritavas ruumis õhkkuivuse saavutamiseni (jääkniiskuseni 18±2%), seejärel purustati proovid nugaveskiga ja sõeluti 1 mm avadega sõelaga. Saadud pulbrist võeti kaks ühe grammist paralleelproovi, mida matsereeriti puhta metanooliga (> 99,9%) suhtes 1:10 72 tundi toatemperatuuril, perioodiliselt loksutades. Seejärel proovid tsentrifuugiti seadmega „Eppendorf 5810R cooling centrifuge" (Eppendorf AG), 15 minutit pööretel 978 *g,* saadud supernatante hoiti analüüsideni -20°C juures (**I**).

Artikli **IV** puhul analüüsiti esmalt 16 rabarberiseemiku juuri ja valiti hüdroksüantrakinoonide sisalduse alusel välja kolm taime, millest kahes oli rohkem hüdroksüantrakinoone (sort ´Victoria´ ja seemik 303) ning üks sort, milles oli vähe hüdroksüantrakinoone (´Ogres´), samadelt taimedelt koguti ka lehevarred (u 200 g). Kõik nimetatud rabarberitaimed kasvavad Polli Aiandusuuringute keskuse aias (58°06´ N, 25°32´ E). Kogutud taimematerjalid lüofiliseeriti, kasutades seadet „VirTis AdVantage 2.0 EL freeze-dryer" (SP Industries) ja säilitati temperatuuril -40°C kuni purustamiseni. Samal viisil toimiti kõigi AB toime ja VRS-i võime võrdluses kasutatud taimsete materjalidega (musta sõstra, söödava kuslapuu ja aroonia marjad ning musta sõstra lehed). Marjad olid katsesse valitud maksimaalse antotsüaanide sisalduse alusel: musta sõstra sort ´Ben Alder´ 37 sordi, söödava kuslapuu sort ´Tomitška´ viie sordi (Sordivaramu, 2021) ja aroonia kolme seemiku hulgast (sordivõrdlusandmed avaldamata). Musta sõstra lehed saadi sordi ´Pamyati Vavilova´ taimedelt, mida ühtlasi koguti tööstuslikuks kasutamiseks, taimede rohkuse tõttu võrreldes teiste sortidega.

Artiklis **II** kasutati musta sõstra lehtede ja marjade polüfenoolse koostise uurimiseks järgnevaid materjale: igalt sordilt (´Karri´, ´Almo´, ´Ats´, ´Elo´, ´Öjebyn´, ´Zagadka´, ´Ben Sarek´, ´Intercontinental´, ´Pamyati Vavilova´, ´Titania´, ´Pilenai´) või seemikult (10B, 2-96-51, 1-96-16, 4-96-1) koguti saagikoristusperioodi keskel 500 g marju kolmel järjestikusel

aastal: 2005, 2006 ja 2007. Lehtede analüüsiks koguti 2009. aasta augusti kuus täielikult välja arenenud lehed kuuelt sordi ´Karri´ põõsalt, võttes lehti võrdselt nii põõsa võra sisemisest kui välimisest tsoonist.

Artiklisse **III** valiti AB ja VRS-i võime hindamiseks alljärgnevad taimsed materjalid: hariliku rabarberi lehevarred ja juured, söödava kuslapuu, tomati, hariliku mustika, hariliku astelpaju ja musta sõstra viljad, millest valmistati tõmmised kahel erineval viisil: jahvatatud lüofiliseeritud taimne materjal, välja arvatud rabarberi juured, mis olid termiliselt kuivatatud mikrobioloogilise saastuse minimeerimiseks, valmistati ette kahel eri meetodil:

A) Dekoktsiooni meetodil puhverdatud veega, vahekorras 1:10 ja kuumutati 95ºC juures 10 min (Bisset ja Wichtl, 1994), saadud lahus jahutati, tsentrifuugiti tsentrifuugiga „Eppendorf 5810R cooling centrifuge“ (Eppendorf AG), tingimustel: 3220 *g*, 10 min. Saadud supernatant tsentrifuugiti uuesti ja lahjendati edasisteks analüüsideks lahusteks 1:20; 1:40 ja 1:80, esialgse droogi suhtes.

B) Kuiv jahvatatud taimne materjal matsereeriti vahekorras 1:10, 30% etanooliga 24 h toatemperatuuril, seda perioodiliselt raputades (18 r/min) pöörleval loksutil Multi RS-60 (BIOSAN). Saadud infusioonid tsentrifuugiti tsentrifuugiga „Eppendorf 5810R cooling centrifuge“ (Eppendorf AG), tingimustel: 3220 *g*, 10 min. Saadud supernatant tsentrifuugiti uuesti ja lahjendati edasisteks analüüsideks lahusteks 1:20; 1:40 ja 1:80, esialgse droogi suhtes (**III**).

Artikli **IV** jaoks matsereeriti lüofiliseeritud ja pulbriks jahvatatud taimne materjal: rabarberi juured ja lehevarred, musta sõstra, söödava kuslapuu ja aroonia marjad ning musta sõstra lehed, üle öö kas 20% või 96% etanooliga, vahekorras 1:20, tsentrifuugiti ja saadud supernatandid hoiti kuni analüüsideni -40°C juures.

Polüfenoolse koostise analüüsideks kasutati 1:20 lahjendusega infusioone, FRS-i toime mõõtmiseks 1:40 lahjenduse ja AB toime mõõtmiseks 1:10, 1:20; 1:40 ja 1:80 (**III**) ning 1:160 (**IV**) lahjendusega infusioone esialgse droogi suhtes.

Töös kasutati taimede polüfenoolse koostise ja polüfenoolide summaarse sisalduse määramiseks (**I, II, IV**) kõrgefektiivset

vedelikkromatograafilist-massspektromeetrilist (*HPLC-DAD-MS*) meetodit, seadmega „1100 Series Agilent 1100 series liquid chromatograph/Mass Selective Detector of ion trap type (LC/MSD Trap)-XCT“ (Agilent Technologies). Polüfenoolsete ühendite tuvastamiseks kasutati elektropihustusionisatsiooniga (*ESI*) ioonlõksu tüüpi massidetektorit negatiivse ja/või positiivse ionisatsiooni režiimil ning UV-*Vis*-detektorit lainepikkuste vahemikus 250–520 nm. Seade koosnes automaatsüstijast, solvendi membraandegaseerijast, binaarsest pumbast, kolonni termostaadist, kolonnist „Agilent Technologies Zorbax 300SB-C18 column“ (150×2,1 mm i.d. osakese suurusega 5 µm) ning UV-*Vis*- ja massidetektorist. Tööd juhiti tarkvara „2D ChemStation Software, ChemStation Spectral SW“ abil. Artiklis **IV** kasutati polüfenoolide üldsisalduse ja eraldi antotsüaanide summaarse sisalduse mõõtmiseks veel ultrakõrgefektiivset vedelikkromatograafi-massispektomeetit „Shimadzu Nexera X2 system“ (Shimadzu Scientific Instruments). Polüfenoolsete ühendite samastamine toimus tunnusainete retentsiooniaegade ja/või ioniseerunud molekulide fragmenteerimisel tekkivate fragmentide võrdlemisel kirjanduse andmetega.

Antioksüdantset toimet hinnati stabiilse vaba radikaali 2,2-difenüül-1-pikrüülhüdrasüüli (DPPH) sidumise meetodil, kasutades „AnalyticJena Specord 200“ spektrofotomeetrit (AnalyticJena AG) koos „WinASPECT“ tarkvaraga. Artiklis **III** kasutati võrdlusainena askorbiinhapet ja artiklis **IV** polüfenoolsete ühendite esindajat − rutiini ehk kvertsetiin-*O*-rutinosiidi.

Antibakteriaalset toimet hinnati agar-kaevu meetodil, viies nelja erineva lahjendusega taimeekstraktid (á 30 µl) bakterisuspensiooniga eelkülvatud agarisse aseptiliselt tehtud avadesse ning inkubeerides seejärel agariplaate vastavatele bakteriliikidele sobivates tingimustes 24 h. Seejärel mõõdeti bakterite kasvu inhibeerivate tsoonide raadiused (**III** ja **IV**). Taimeekstraktide AB toime hindamisel kasutati võrdlusainena naatriumnitritit ja positiivse kontrollina klooramfenikooli vesilahust 1000 mg/l. Lihatööstuses saavutatakse AB toime siiski naatriumnitriti lisamise ja teiste tehnoloogiliste protsesside (kuumtöötlemine, pH vähendamine, vinnutamine jms) kasutamise, mitte antibiootikumide lisamisega.

Rabarberi ja musta sõstra polüfenoolse koostise määramise tulemused

Hariliku rabarberi juurtes leiti artiklis **I** kasutatud materjalis erinevaid resveratrooli, deoksürapontigeniini, rapontigeniini, pitseatannooli, krüsofanooli, emodiini, torakrüsooni, pterostilbeeni ja aaloe-emodiini derivaate (**I**).

Rabarberi vartes leiti lisaks seitse erinevat flavonooli, mis olid kvertsetiini ja müritsetiini derivaadid (**I**). Artiklis **IV** leiti rabarberi vartes positiivse ionisatsiooni režiimi ja UV-*Vis*-detektori abil (520 nm) ka antotsüaane. Artiklis **IV** uuriti mitut erinevat hariliku rabarberi seemikut (16 varem analüüsitud seemiku hulgast valiti kolm), mille puhul selgus, et nende polüfenoolne koostis erines kvalitatiivselt küllaltki palju. Tulemus kinnitab taas, et ühe liigi piires võib esineda sorte või seemikuid, millel on erinev kemotüüp ja sellest lähtuvalt võib esineda ka erinev AB toime või VRS-i võime. Rabarberiekstraktide polüfenoolsete ühendite profiil sõltus ka solvendi valikust. Hüdrofoobsemad ühendid, mis ekstraheerusid 96% etanooliga, on töö inglisekeelses osas Tabelis 3 märgistatud tärniga.

Musta sõstra lehtedes leitud polüfenoolsed ühendid (peamiselt fenoolsete hapete ja flavonoidide rühmadesse kuuluvad ühendid) on välja toodud artiklites **II** ja **IV**: katehhiingallaat, klorogeenhape I, dihüdrofeerulhappe ramnosiid, klorogeenhape II, feerulhappe derivaat, kumaroüülkviinhape, kumaroüülkviinhappe pentosiid, müritsetiini glükosiid, kvertsetiin-3-rutinosiid (rutiin), kvertsetiini glükosiid, kvertsetiini atsetüülglükosiid, kamferooli rutinosiid, kamferool-3-*O*-glükosiid, kamferooli atsetüülglükosiid, isoramnetiini atsetüülglükosiid, ja krüsofanooli glükosiid.

Musta sõstra marjades leiti fenoolseid happeid: klorogeenhapet, kohvhappe-*O*-glükosiidi, kumarüülkviinhapet; antotsüaane: delfinidiin-3-*O*-glükosiidi, delfinidiin-3-*O*-rutinosiidi, tsüanidiin-3-*O*-glükosiidi, tsüanidiin-3-*O*-rutinosiidi; ja teisi flavonoide: isoramnetiin-3-*O*-rutinosiidi, müritsetiin-*O*-glükosiidi ja rutiini (**IV**). Leitud polüfenoolne profiil on sarnane Vagiri jt (2012) analüüsitulemustega. Hiljutises uurimustöös on Tian jt (2019) leidnud musta sõstra marjadest veel erinevaid polüfenoolseid ühendeid, eriti antotsüaane, mis võib tuleneda täpsema analüüsimeetodi ja -tehnika kasutamisest, aga ka erinevustest

proovi ettevalmistusel. Seega võib järeldada, et käesolevas töös leitud polüfenoolse koostise nimekiri pole ei hariliku rabarberi ega musta sõstra erinevate taimeosade puhul kaugeltki lõplik. Taime kasvuperioodi eri aegadel ning ka proovi töötlemisel ja säilitamisel võib polüfenoolne koostis muutuda. Samuti võib liigi piires esineda erinevaid kemotüüpe. Lisaks võimaldavad erinevad analüüsiseadmed ja vastava taime jaoks loodud sobiv kromatograafiline voolutigradient leida proovist rohkem erinevaid polüfenoolseid ühendeid. Põhjalikumaks taimede kvalitatiivseks analüüsimiseks võib olla vajalik iga taimeliigi jaoks eraldi välja töötada kromatograafiline gradient ja uurida taimeliigi kasvuaja vältel eri aegadel kogutud proove. Üks pikk universaalne gradient on omal kohal, kui soovida analüüsida eri taimeliikide polüfenoolse profiili sarnasusi. Samuti tuleb taimeproovidest erinevate ühendite ekstraheerimiseks kasutada mitut erinevat solventi.

Taimse materjali edasisel kasutamisel mõnes teises toidumaatriksis antimikroobse või antioksüdantse toime saavutamiseks peaks taimne materjal olema eelnevalt standarditud mõne tugevama sel viisil toimiva polüfenoolse või muu ühendi suhtes. Selleks on vajalik läbi viia täpsemad uuringud, et selgitada välja, millised ühendid põhjustavad erinevates taimedes vastavaid toimeid.

Antibakteriaalse toime analüüside tulemused

Väitekirja aluseks olevate artiklite **III** ja **IV** tulemused kinnitavad, et taimedest 96%-lise etanooliga tehtud tõmmistel on tugevam antibakteriaalne toime kui madalama etanooli sisaldusega (20% või 30%) lahusega või veega saadud tõmmistel. Katsetes kasutatud taimedest avaldasid söödava kuslapuu ja astelpaju marjad ning rabarberi varred antibakteriaalset toimet sellistele toidupatogeenidele nagu *L. monocytogenes*, *E. coli* või *C. jejuni*, sealjuures oluliselt pidurdamata probiootiliste bakterite, eriti *B. bifidum* kasvu. Seega neid taimeosi võib katsetada ka nt probiootilistes piimatoodetes neile lisaväärtuse andmiseks. Analüüsitud taimeosadest avaldasid kõige tugevamat AB toimet rabarberijuurtest (**III** ja **IV**) etanooliga valmistatud infusioonid, mida ei saa aga veel soovitada toidus kasutamiseks, sest pole läbi viidud rabarberijuure ohutust tõendavaid riskianalüüse. Rabarberi vartest ja mustsõstra marjadest 96% etanooliga valmistatud infusioonid avaldasid samuti AB toimet kõigi uuritud bakteriliikide suhtes (**IV**).

Kuigi artiklis **III** leiti, et uuritud taimeekstraktid olid uuritud bakteriliikide suhtes tugevama AB toimega kui naatriumnitriti vesilahus, siiski ei soovita artikli autorid lihatoodetesse naatriumnitriti lisamist täielikult taimsete lisanditega asendada. Selliste soovituste andmiseks on vajalik läbi viia põhjalikumad teadusuuringud, mis arvestaksid eriti *Clostridium botulinum*'i ja teiste klostriididega seotud ohte.

Vabade radikaalide sidumise võime analüüside tulemused

Artiklis **III** uuritud taimeosadest puhverdatud kuumutatud veega valmistatud infusioonid avaldasid kõik tugevamat VRS võimet kui samades tingimustes valmistatud askorbiinhappe lahus kontsentratsiooniga 1 mg/ml. Selgus, et proovi kuumutamine enne analüüsi põhjustas askorbiinhappe sisalduse languse ja seetõttu ka VRS võime nõrgenemise võrreldes toatemperatuuril 30%-lise etanooliga valmistatud lahustega, kus askorbiinhappe sisaldus paremini säilis. Teine põhjus, miks 30%-lise etanooliga valmistatud infusioonidel leiti tugevam VRS toime võib olla asjaolu, et etanooliga ekstraheerub taimsest materjalist rohkem hüdrofoobseid ühendeid, millel võivad olla nii VRS võime kui ka AB toime. VRS võime tugevuse järgi reastusid artiklis **III** uuritud taimeosadest puhverdatud vette valmistatud infusioonid, alates tugevaimast, järgnevalt: söödava kuslapuu marjad; mustsõstra marjad; askorbiinhappe lahus (10 mg/ml), astalpaju marjad, rbarberi varred, mustika marjad, tomati viljad, askorbiinhappe lahus (1 mg/ml) ja rabarberi juured. Tulemusi saab eelteadmisena aluseks võtta selleks, et edaspidi süsteemsemalt hinnata, millised taimed sobiksid kasutada parema VRS võime saavutamiseks kuumutatud toiduainetes. Samas reastusid 30%-lises etanooli lahuses toatemperatuuril valmistatud infusioonid tugevaimast alustades järgnevalt: askorbiinhappe lahus (10 mg/ml), rabarberi varred, mustsõstra marjad, mustika marjad, rabarberi juured, söödava kuslapuu marjad, askorbiinhappe lahus (1 mg/ml), astelpaju marjad ja tomat.

Kasutatud VRS võime mõõtmise meetodil, DPPH radikaali sidumise kaudu, on puuduseks see, et sinised marjad neelavad valgust DPPH-ga samal lainepikkusel. Selle efekti mõju saab vähendada kui marjalahuseid piisavalt palju lahjendada, et nende värvus ei segaks reaktsiooni jälgimist. Käesolevas töös lahjendati esialgseid taimelahuseid neli kuni kuusteist korda, selleks, et saaks reaktsiooni mõõta ning samuti piisavalt selleks, et tulemusi võrrelda askorbiinhappe (**III**) või rutiini (**IV**) kalibratsiooni

lahuste lineaarses alas toimunud mõõtmistega. Tulemused näitasid, et VRS võime kuumutatud vesilahustes oli, ilmselt kuumutamise mõju tõttu, tugevas positiivses korrelatsioonis askorbiinhappe sisaldusega (r = 0,94) **III**, aga etanooli lahustes oli seos nende kahe parameetri vahel väga nõrk (r < 0,1) (**III**) või nõrk kuni keskmine (r = 0,4−0,64) (**IV**). Etanooli ekstraheeruvad paremini polüfenoolsed ühendid (Villano jt, 2007), eriti antotsüaanid (Chaovanalikit jt, 2004; Viljanen jt, 2005; Paśko jt, 2009), millel samuti on VRS võime. Käesolevas töös uuritud taimedest leiti kõrgeimad antotsüaanide sisaldused aroonia marjades (**IV**) ja söödava kuslapuu marjades (**III**, **IV**). Sealjuures oli kõrge antotsüaanide sisaldusega marjadel tugev VRS võime, mis kinnitab varasemate uurimuste tulemusi (Viljanen jt, 2005, Paśko jt, 2009 ja Tian jt, 2018). Kõrgeim polüfenoolide kogusisaldus leiti rabarberi juurtes (**III**, **IV**), aga samas oli rabarberi juurte vesilahuse VRS võime üks madalaimaid. Kui aga vaadata rabarberi varte tugevat VRS võimet, võrreldes isegi kõrge antotsüaanide sisaldusega marjadega ja sealjuures nende üldiselt madalat polüfenoolsete ühendite sisaldust, siis peab tõdema, et lisaks antotsüaanidele ja mõnedele teistele polüfenoolsetele ühenditele ja askorbiinhappele peab edaspidi tähelepanu pöörama ka veel muudele ühenditele taimedes, millel võib VRS võime esineda.

Artikli **IV** tulemuste järgi olid aroonia marjad, mustsõstra marjad ja söödava kuslapuu marjad väga sarnaste VRS omadustega. Üle kõigi uuritud taimede oli VRS võime positiivses korrelatsioonis antotsüaanide sisaldusega nii 20%-liste etanooli lahuste (r = 0,65) kui ka 96%-liste etanoolilahuste puhul (r = 0,47). Keskmises positiivses korrelatsioonis oli VRS tvõime askorbiinhappe ja sidrunhappe sisaldusega.

Taimelahuste AB toime ja VRS võime ei olnud omavahel tugevas positiivses korrelatsioonis, mis viitab sellele, et neid toimeid põhjustavad osaliselt erinevad ühendid.

Tuginedes varasematele töödele, Villano jt (2007) ja Csepregi jt (2016), võib välja tuua mõnede polüfenoolsete ühendite VRS võime tugevuse järjekorra, võrdluses askorbiinhappega: tsüanidiin ~ delfinidiin ~ kvertsetiin ~ müritsetiin > katehiin > gallushape > rutiin > malvidiin ~ pelargonidiin > askorbiinhape > *trans*-resveratrool > *trans*-rapontiin > emodiin.

Järeldused ja soovitused

1. Uuritud taimeekstraktide AB toime ei olnud tugevas positiivses korrelatsioonis VRS-i võimega, millest võib järeldada, et neid toimeid põhjustavad erinevad ühendid. Seega tuleks mõlema toime saavutamiseks omavahel kombineerida tugeva AB ja VRS-i võimega taimi. Antud töö kontekstis sobiksid selleks kombineerituna rabarberi varred ja mõned antotsüaane ja/või rikkalikult C-vitamiini sisaldav marjad (aroonia, söödav kuslapuu, mustsõstar).

2. Kõigist uuritud taimeekstraktidest oli kõige tugevama AB toimega rabarberijuurtest 96% etanooliga valmistatud ekstrakt. Seega võib rabarberijuurt esile tõsta kui heade antibakteriaalsete omadustega taime, mida ei saa aga veel soovitada toidus kasutamiseks, sest pole läbi viidud rabarberijuure ohutust tõendavaid riskianalüüse.

3. Söödava kuslapuu, hariliku mustika, musta sõstra ja astelpaju marju võib soovitada kasutamiseks toiduainetööstuses mh probiootiliste toodete koostises, sest nendest marjadest valmistatud lahustel oli tugev VRS-i võime, aga nad ei pärssinud sealjuures probiootiliste bakterite kasvu. Muidugi peaksid toidule lisatavad taimekogused ja lisamisviisid olema sensoorselt vastuvõetavad ja mikrobioloogiliselt ohutud.

4. Vabade radikaalide sidumisvõime hindamisel ilmnes, et taimeekstrakte tuleb vastavalt liigile piisavalt lahjendada, et mõõdetavad tulemused oleksid võrdlusaine kontsentratsiooni suhtes lineaarses alas.

5. Kuigi artiklis **III** leiti, et uuritud taimeekstraktid olid uuritud bakteriliikide suhtes tugevama AB toimega kui naatriumnitriti vesilahus, siiski ei soovita artikli autorid lihatoodetesse naatriumnitriti lisamist täielikult taimsete lisanditega asendada. Selliste soovituste andmiseks on edaspidi vajalik läbi viia põhjalikumad teadusuuringud, mis arvestaksid eriti *Clostridium botulinum*'i ja teiste klostriididega seotud ohte.

6. Käesoleva töö valmimise käigus ei leitud põhjalikku toidutaimede toitainelise koostise andmebaasi. Eriti tuntav oli orgaaniliste hapete nimekirja puudujääk kasutatud andmebaasides: FINELI (2021),

„USDA Food Composition Database“ (2019) ja toidu koostise andmebaas NutriData (2021). Söödava kuslapuu marjade toitaineline koostis puudus neis andmebaasides. Ka läbitöötatud teaduskirjanduse abil ei õnnestunud puuduvaid lünki täielikult täita, mistõttu on analüüsitud toidutaimede toitainelise koostise kirjeldused ebatäielikud. Vajadus on eelmainitud toitainete sisaldusi kajastava põhjaliku andmebaasi järele, mida saaks kasutada näiteks nii menüüarvutustes kui ka statistiliste analüüside läbiviimiseks

7. AB ja VRS omadused sõltuvad eri taimeliikide puhul erinevatest polüfenoolsetest jm ühenditest. Edasistes uuringutes on vajalik analüüsida, millised polüfenoolsed jm ühendid mõjutavad eri taimsete materjalide puhul nii antioksüdantset kui ka antibakteriaalset toimet, kui neid lisada erinevatesse toidumaatriksitesse. Taimne materjal tuleks ka nende ühendite suhtes standardida, et saavutada igal kasutamisel võrdväärne toime.

8. Teema edasisel uurimisel tuleks süsteemsemat tähelepanu pöörata veel näiteks järgmistele aspektidele:

    a) Taimeliikide vaheliste AB toime ja VRS-i võime erinevuste välja selgitamisele, võrreldes paarikaupa erinevaid taimi, sealjuures suurendades proovide korduste arvu.

    b) Ekstraktsiooniaja optimeerimisele, et säiliks maksimaalne AB ja VRS võime.

    c) Taimse materjali kuumutamise mõjule, seoses AB toime ja VRS-i võimega.

    d) Uuritud taimede võrdlemisele mõnede sagedamini kasutatavate maitse ja vürtsitaimedega, nagu rosmariin, oregano, küüslauk jt.

10. ACKNOWELEDGEMENTS

I am most grateful to my supervisors, Professor Tõnu Püssa, PhD and senior rsearcher Ave Kikas, PhD for advice and moral support throughout the years of my studies. My equal gratitude belongs to my earlier academic supervisors: Professor Ain Raal, PhD and Ülle Reier, PhD from University of Tartu and to Professor Kadri Karp, PhD from Estonian University of Life Sciences.

Many thanks to all of my colleagues from the Chair of Food Hygiene and Veterinary Public Health, especially to Professor Mati Roasto, PhD, Dea Anton, Katrin Laikoja and Kadrin Meremäe, PhD for creating a working environment, where everybody feels oneself as an essential part of the team.

I thank all of my coauthors of the papers, published during the writing of this thesis.

I am grateful to Līga Lepse, PhD from the the Latvian Institute of Horticulture; and to Hans Neppo, Liina Arus, PhD, Hedi Kaldmäe and Kersti Kahu from Polli Horticultural Research Centre, Estonia for providing the plant material for the research. I am grateful to Peeter Laurson from the Competence Centre "PlantValor" for performing the Fourier Transform Near-Infrared (FT-NIR) spectrometry analyses for paper **IV** and for directing me to focus on summing things up. I thank Professor Tanel Kaart, PhD for some valuable suggestions, regarding my work; Professor Rainer W. Bussmann, PhD for explaining the botanical names of rhubarbs; Priit Pedastsaar for performing the statistical analyses with R-software and EMU IT-specialist Taavo Vellend for updating my laptop, so I could write my thesis.

I am grateful to Urve Ansip and the University terminology committee for the Estonian linguistic corrections and to Mrs. Ursula Erik and Mrs. Ingrid Helvi Williams for English revision of the thesis. I wish to thank Kerli Mõtus, PhD for proofreading and Alo Tänavots, PhD for his aid in formatting the manuscript.

I am grateful for the libraries of University of Tartu and Estonian University of Life Sciences, where I have spent many hours during my studies and writing of this thesis.

I am grateful to the local government of Island of Muhu for supporting my academic life in its early stage, also to SA Archimedes, COST programme and the Doctoral Schools of Estonian University of Life Sciences for supporting financially my participation in different conferences and doctoral schools abroad (Ireland, Spain, Germany and Finland), together with the following funding sources:

1) Estonian Science Foundation (research grants no. 6801 and 7703)

2) Ministry of Education and Research of Estonia (target financing project no. 1092711s06)

3) European Community's Regional Development Fund in the Framework of the Competence Centre Programme of the Enterprise Estonia under project No European Union (EU) 30002 of Bio-Competence Centre of Healthy Dairy Products;

4) Estonian Research Council (Sihtasutus Eesti Teadusagentuur, ETA-g) Grant no. 9315EU

5) ERA-NET SUSFOOD International Project: „Sustainable plant ingredients for healthier meat products – proof of concepts" (SUSMEATPRO)

6) The Base funding project of Estonian University of Life Sciences P170054VLTH: „Metabolomics, antioxidative and antibacterial activity, and related mechanisms of food plants"

7) The Base funding project of Estonian University of Life Sciences P180279VLTR: "The study of the effect and related mechanisms of natural bioactive substances in food matrices"

Finally, I am grateful to the dear members of my family, friends, teachers, colleagues, who have been there for me, at different times of my life. I am sorry that I am often preoccupied with work, but I hope to be there for you too.

I

ORIGINAL PUBLICATIONS

Püssa, T., **Raudsepp, P**., Kuzina, K., Raal, A., 2009.
POLYPHENOLIC COMPOSITION OF ROOTS AND
PETIOLES OF *RHEUM RHAPONTICUM* L.
Phytochemical Analysis. 20, 98−103.
doi: 10.1002/pca.1102

Research Article

Phytochemical Analysis

Received: 3 October 2007, Revised: 26 August 2008, Accepted: 2 September 2008 Published online 31 October 2008 in Wiley Interscience

(www.interscience.wiley.com) DOI 10.1002/pca.1102

Polyphenolic composition of roots and petioles of *Rheum rhaponticum* L.

Tõnu Püssa,[a] Piret Raudsepp,[a] Kristina Kuzina[b] and Ain Raal[b]*

ABSTRACT:
Introduction – Various species of the genus *Rheum* (*Polygonaceae*) are known for their high content of medicinally important hydroxyanthraquinones. However, little information is available concerning the polyphenolic composition of garden or dietary rhubarb *Rheum rhaponticum* L. (*R. rhaponticum*).
Objective – Determination of further polyphenols in the roots and petioles of *R. rhaponticum*.
Methodology – The dried plant material was extracted with 10-fold excess (v/w) of methanol and subsequently diluted five times with methanol–water (1:1) and analysed by reversed-phase liquid chromatography using tandem UV-photodiode array and mass selective detection (RP-HPLC-UV-ESI/MS^2). Polyphenols were identified using either HPLC-ESI/MS^2 data obtained for respective commercial standards or by comparison of a parent ion fragmentation picture with the respective MS^2 spectrum from the literature.
Results – The roots of R. *rhaponticum* were very rich in various hydroxystilbenes and contained four main substance groups – derivatives of *trans*-piceatannol, *trans*-resveratrol, *trans*-rhapontigenin and *trans*-deoxyrhapontigenin. Additionally, pterostilbene acetylglucosides and a number of hydroxyanthraquinones and their glycosides were identified in the root samples. The profile of polyphenols in the petioles of *R. rhaponticum* was similar to that of the roots but the content of individual substances was remarkably lower. The petioles of the *R. rhaponticum* additionally contained significant amounts of derivatives of flavonol quercetin, which is a good antioxidant.
Conclusion – The study has shown that roots of *R. rhaponticum* contain a wide variety of hydroxystilbenes and deserve further consideration as a source of medicinally interesting compounds.

Keywords: HPLC-UV-ESI/MS^2; polyphenols; hydroxystilbenes; hydroxyanthraquinones; *Rheum rhaponticum*; garden rhubarb

Introduction

Different species of the genus *Rheum* (*Polygonaceae*), such as *R. palmatum* L., *R. emodi* Wall. ex Meisn. and *R. officinale* Baill. are known for their high content of physiologically active hydroxyanthraquinones (aloe-emodin, emodin, chrysophanol, rhein, physcion and their glycosides) in their roots (Okabe *et al*., 1973; Agarwal *et al*., 2000, Komatsu *et al*., 2006). On the other hand, a number of hydroxystilbenes and their derivatives have been identified in the rhizome of Korean rhubarb *R. undulatum* L. (Kashiwada *et al*., 1984; Ko, 2000; Matsuda *et al*., 2000a,b; Kageura *et al*., 2001), in the roots of *R. maximowiczii* Loinsk (Shikishima *et al*., 2001) and in the rhizome of *R. emodi* (Babu *et al*., 2004). More than 100 different phenolic compounds have either been identified or tentatively characterised in six rhubarb species (*R. officinale*, *R. palmatum*, *R. tanguticum*, *R. franzenbachii*, *R. hotaoense* and *R. emodi*) by their parent ion fragmentation spectra by Ye and co-authors (Ye *et al*., 2007).

There is substantially less data in the literature on the content of phenolic compounds in the roots and especially in petioles of garden or dietary rhubarb *R. rhaponticum* L., which in many aspects is similar to *R. undulatum*. *R. rhaponticum*, the petioles of which are used as a food component, has been known in Chinese ethnopharmacology from ancient times. It has been shown that the rhizomes of this rhubarb contain hydroxystilbene rhaponticin (synonyms rhapontin and rhapontigenin glucoside), as well as its precursor *trans*-resveratrol (Rupprich *et al*., 1980). The latter is widely known for its versatile therapeutic potential (Piver *et al*., 2003; Baur and Sinclair, 2006). The physiological properties of resveratrol oligomers and related hydroxystilbenes have been much less studied. Resveratrol oligomers usually support the action of the monomer, but they may act also in a different manner (Piver *et al*., 2003). For example, it has been demonstrated that resveratrol oligomers strongly suppress HL-60 cell proliferation and induce DNA damage (Kang *et al*., 2003). Rhaponticin or rhapontin elicits antithrombotic and antiallergic properties (Park *et al*., 2002) and isorhapontigenin attenuates cardiac hypertrophy (Li *et al*., 2005).

A special extract from the roots of *R. rhaponticum*, referred to as ERr 731® (trade name Phyto-Strol®, Carl Müller, Göppingen, Germany), has been regularly prescribed for climacteric complaints since 1993, and has been used in Germany for decades for women of child-bearing age suffering from oligomenorrhoea or amenorrhoea. According to recent investigations, the main active components in this extract are hydroxystilbenes (Möller *et al*., 2007; Wober *et al*., 2007).

A limited number of references can be found concerning the determination of complex glycosides such as *O*-acetyl- or *O*-galloylglucosides of hydroxystilbenes or hydroxyanthraquinones in plants (Okasaka *et al*., 2004; Komatsu *et al*., 2006; Ye *et al*.,

* Correspondence to: A. Raal, Institute of Pharmacy, University of Tartu, Nooruse 1, 50411 Tartu, Estonia. E-mail: ain.raal@ut.ee

[a] Department of Food Science and Hygiene, Estonian University of Life Sciences, Kreutzwaldi 58A, 51014 Tartu, Estonia

[b] Institute of Pharmacy, University of Tartu, Nooruse 1, 50411 Tartu, Estonia

2007). The two first-mentioned papers present the results of resveratrol derivatives.

Quercetin-3-*O*-rutinoside (rutin), -3-*O*-glucoside and -3-*O*-rhamnoside (quercitrin), as well as 1-glucose esters of ferulic, sinapic and *p*-coumaric acids have been identified in a non-specified part of *R. rhaponticum* (Blundstone, 1970).

Earlier we published the preliminary outcomes of a study of hydroxystilbenes of *R. rhaponticum* (Aaviksaar *et al*., 2003). Here we present the results of a more comprehensive investigation of polyphenolic composition of *R. rhaponticum* using reversed phase liquid chromatography with a tandem UV–photodiode array and mass selective detection (HPLC-UV-MS^2). The main objectives of the present study were to identify and semiquantify of possibly more different polyphenols in the roots and petioles of *R. rhaponticum*.

Experimental

Materials. Samples (batches of 50 g each) of roots and petioles of *R. rhaponticum* were collected monthly from June to October, 2003 and from May to October, 2004 near Kuusalu in the Northern Estonia. Voucher specimens (Nooruse 1, Tartu 50411, Estonia, no. 715) of the rhubarb roots and petioles are deposited at the Institute of Pharmacy, University of Tartu, Estonia. After cutting into pieces with maximum dimensions 1×5 cm, the plant material was dried for 10 days at room temperature in the absence of light in a well-ventilated room. The air-dried samples with a residual moisture content of $18 \pm 2\%$ were powdered in a mortar, the powder sieved through a 1 mm sieve and two parallel sub-samples comprising 1 g each were macerated with a 10-fold excess (v/w) of methanol (>99.9%) for 72 h at room temperature with periodic shaking. Following centrifugation in an Eppendorf AG (Hamburg, Germany) model 510R cooling centrifuge, equipped with a swinging bucket rotor for 15 min at 978 *g*, the supernatants were kept at −20°C. After removal of the supernatant and short-term rinsing with 2 mL of water, the sediment was re-extracted with another 10 mL of methanol. The chromatographic analysis of both extracts showed that the first extracts, which were used for further investigation, contained 90–95% of various phenolic constituents of the plant samples.

Commercial standards of resveratrol, piceatannol, rhapontin, deoxyrhapontin, aloe-emodin, emodin, rhein, quercetin, rutin and myricetin were from Sigma Chemical Company, St. Louis, USA; physcion was from Fluka Chemie GmbH, Buchs, Switzerland; piceid (polydatin) was from Chromadex Inc. (Irvine, CA, USA); and astringin from Polyphenols Laboratories AS (Sandnes, Norway). All solvents were of HPLC-grade and purchased from Romil (Cambridge, UK), and formic acid of MS-grade was from Fluka.

Chromatographic equipment. For the identification of rhubarb polyphenols, the HPLC-UV-MS^2 analyses were performed on an 1100 Series LC/MSD Trap-XCT (Agilent Technologies, Palo Alto, CA, USA) equipped with an electrospray interface (ESI). The ion trap, working in the negative ionisation mode, was connected to an Agilent 1100 Series HPLC instrument consisting of an autosampler, solvent membrane degasser, binary pump, column thermostat and a UV–vis photodiode array detector. The HPLC 2D ChemStation Software with a ChemStation Spectral SW module was used for process guidance.

Chromatographic conditions. The reversed-phase-HPLC analytical separation was performed on an Agilent Technologies Zorbax 300SB-C_{18} column (150×2.1 mm i.d.; 5 μm particle size) with a guard column filled with the same type of sorbent, in a stepwise gradient mode of 0.1% formic acid (solvent A) and acetonitrile (solvent B) at a flow rate of 0.3 mL/min at 35°C. Elution was started with a linear gradient of B from 10 to 30% at 30 min, then to 90% at 40 min and finished isocratically with 90% of B for 10 min.

For the liquid chromatographic analysis, the extract supernatants were diluted five times with methanol–water (1:1 v/v) and 10 μL of the solution obtained was injected into the chromatographic system without further purification. The UV photodiode-array detector recorded in the interval 200–600 nm and the eluate optical density (OD) was continuously monitored at wavelengths 250 (phenolic acids), 280 (flavanols), 306 (*trans*-hydroxystilbenes), 370 (flavonols) and 430 (hydroxyanthraquinones) nm. The conditions of MS^2 detection were: *m/z* interval 50–1000; target mass, 400; number of fragmented ions, two; maximal accumulation time, 100 ms; compound stability, 100%; drying gas (nitrogen from generator) flow rate, 10 L/min; gas temperature, 350°C; nebuliser pressure, 30 psi; collision gas He pressure, 6×10^{-6} mbar.

Results and Discussion

Identification of polyphenols

Polyphenols were identified using either HPLC-MS^2 data obtained from respective commercial standards analysed in parallel chromatographic runs or by comparison of the parent ion fragmentation picture with the respective MS^2 spectrum from literature. Sample $UV_{306\ nm}$ and MS-base peak chromatograms (BPC) of root extracts of the rhubarb are presented in Fig. 1.

All the polyphenolic compounds, hydroxystilbenes, -anthraquinones and –naphthalenes, determined by HPLC-MS^2 in the negative ionisation mode together with their MS^2 characteristics and heights of the extracted ion chromatographic (EIC) peaks in the diluted extract of the roots and petioles from June 2003 are listed in Table 1. The respective data for the flavonols of the petioles are presented in Table 2. Since the chromatograms of different samples were rather similar to each other, in Table 1 only the results from June 2003 are presented as the most representative. Semi-quantitative assays were carried out when the signal-to-noise ratio (S/N) of the particular peak was at least 5. In all 25 hydroxystilbenes and their various derivatives, eight hydroxyanthraquinones and their *O*-glycosides, two *O*-glycosides of hydroxynaphthalenes and seven flavonols and their derivatives were identified and semi-quantified in the roots and petioles of *R. rhaponticum*. All of these substances were present in the methanol extract of *R. rhaponticum* roots, in which altogether seven derivatives of resveratrol, six of piceatannol and rhapontigenin, four of deoxyrhapontigenin, three of chrysophanol and emodin, two of torachrysone and pterostilbene and one of aloe-emodin were identified (Fig. 2). The identification of polyphenols was carried out as follows.

Aglycones. *trans*-Resveratrol, *trans*-piceatannol and emodin were identified by comparison of their MS^2 spectra with the fragmentation spectra of the respective commercial standards, and *trans*-deoxyrhapontigenin by comparison of its MS^2 spectrum with the respective spectrum of the commercial standard of deoxyrhapontin.

Table 1. List of hydroxystilbenes, -anthraquinones and -naphthalenes identified in the roots and the petioles of *R. rhaponticum*

Compound	t_R, min	$[M-H]^-$	*m/z* of the main daughter ions (in parentheses the relative intensities—intensity of the first, most abundant, ion is taken as 100)	Height of EIC peak[a]	
				Petioles	Roots
Piceatannol *O*-glucoside 1	7.4	405	**243**; 225 (0.5); 201 (0.5); 199 (0.5); 159 (0.3); 173 (0.3)	405	n.d.
Piceatannol *O*-glucoside 2 (astringin-according to the commercial standard)	8.3	405	**243**; 225 (1); 201 (0.8); 228 (0.3); 159 (0.2); 157 (0.2)	202	17,079
Resveratrol *O*-glucoside 1 (putative resveratroloside)	8.6	389	**227**; 185 (1.0); 179 (1.0); 269 (1.0); 143 (0.9)	1884	39,164
Resveratrol *O*-glucoside 2 (piceid)	11.4	389	**227**; 185 (2.2); 251 (1.8); 269 (1.0); 157 (0.5); 143 (0.5)	189	8680
Piceatannol *O*-galloylglucoside 1	12.4	557	**243**; 405 (60); 313 (35); 441 (6); 169 (4); 201 (2)	n.d.	2850
Piceatannol[b]	13.2	243	**175**; 225 (60); 149 (52); 215 (26); 181 (24); 201 (20)	n.d.	1512
Piceatannol *O*-galloylglucoside 2	14.1	557	**313**; 243 (39); 405 (29); 169 (6); 395 (4)	61	7043
Rhapontigenin *O*-glucoside (rhapontin) 1[b]	15.0	419	**257**; 241 (3.3); 281 (1.8); 299 (1); 401 (0.4); 225 (0.2)	5829	56,073
Resveratrol *O*-galloylglucoside	16.7	541	**313**; 169 (7.2); 227 (3.3); 379 (2.0); 241 (1.6); 389 (1.4)	628	28,593
Rhapontigenin *O*-glucoside 2 (rhapontin 2)[b]	18.1	419	**257**; 241 (4.5); 172 (2.5); 299 (1.5); 281 (1.0); 143 (0.5)	847	5176
trans-Resveratrol [b]	18.3	227	**185**; 183 (49); 157 (43); 159 (26); 143 (18); 213 (9); 209 (5)	87	2901
Rhapontigenin *O*-galloylglucoside	19.0	571	**313**; 556 (15); 169 (11); 257 (6); 327 (4); 409 (3); 419 (1)	417	20,027
Rhapontigenin *O*-acetylglucoside 1	19.3	461	**299**; 257 (53); 401 (48); 419 (40); 357 (20); 231 (8); 213 (6)	85	4544
Aloe-emodin *O*-glucoside[c]	20.0	431	**269**; 293 (2.1); 311 (1.7); 225 (1.2); 240 (1.1); 283 (1.0)	328	7923
Rhapontigenin	21.8	257	**242**; 241 (60); 224 (11); 172 (5)	64	8084
Rhapontigenin *O*-acetylglucoside 2	22.1	461	**257**; 401 (7.1); 299 (6.0); 241 (5.1); 419 (2.7)281 (1.9)	n.d.	3121
Deoxyrhapontigenin *O*-glucoside (deoxyrhapontin)[b]	24.2	403	**241**; 265 (3); 226 (0.7); 161 (0.7); 283 (0.5)	8762	43,033
Torachrysone *O*-glucoside[d]	25.4	407	**245**; 230 (2.1); 215 (0.5)	671	23,205
Chrysophanol *O*-glucoside[c]	25.6	415	**253**; 225 (4); 295 (3.7); 277 (3.2); 175 (2.9); 267 (2.5)	131	11,807
Emodin *O*-glucoside[c]	25.8	431	**269**; 293 (6.0); 311 (7.4); 413 (2.9); 225 (1.5)	920	8027
Deoxyrhapontigenin *O*-galloylglucoside	27.6	555	**313**; 169 (13); 241 (3.2); 393 (1.3); 211 (1.0)	n.d.	17,955
Deoxyrhapontigenin *O*-acetylglucoside	28.2	445	**241**; 225 (2); 385 (1.7); 265 (1.5)	95	4101
Pterostilbene *O*-acetylglucoside 1	28.7	459	**255**; 227 (19); 399 (15); 209 (8); 181 (1)	n.d.	763
Torachrysone *O*-acetylglucoside[d]	28.8	449	**245**; 389 (15); 230 (14); 403 (5); 215 (2)	64	1553
Resveratrol dimer 1	29.4	453	**359**; 347 (34); 435 (22); 369 (15); 411 (12); 333 (6); 289 (5)	31	14,562
Emodin *O*-malonylglucoside[c]	30.0	517	**473**; 431 (1.5); 269 (1); 293 (1); 311 (1)		
Pterostilbene *O*-acetylglucoside 2	30.4	459	**255**; 227 (13); 209 (5); 181 (1)	105	6400
Chrysophanol *O*-acetylglucoside 1[c]	30.4	457	**253**; 225 (14); 207 (7); 209 (3); 165 (2); 155 (2); 295	n.d.	n.d.
Rhein *O*-glucoside[c]	30.6	445	**283**; 269 (1.0); 325 (0.9); 307 (0.8); 297 (0.5)	97	n.d.
Chrysophanol *O*-acetylglucoside 2[c]	31.4	457	**253**; 225 (16); 209 (2); 165 (2); 207 (1); 235 (1); 295	n.d.	n.d.
Deoxyrhapontigenin	33.2	241	**226**; 225 (62); 197 (4); 213 (3); 195 (3); 175 (2)	n.d.	5210
Resveratrol dimer 2	35.4	453	**435**; 369 (88); 411 (70); 409 (35); 333 (32); 347 (18); 367 (11)	42	21,877
Resveratrol dimer 3	36.4	453	**435**; 369 (77); 333 (68); 411 (64); 409 (32); 359 (28); 367 (25)	43	1341
Resveratrol dimer 3	36.4	453	**435**; 369 (77); 333 (68); 411 (64); 409 (32); 359 (28); 367 (25)	43	1341
Emodin[c], [b]	39.5	269	**225**; 241 (24); 210 (6); 151 (3); 181 (2)	7758	9395

[a] The numbers (in mAU $\times 10^{-3}$) belong to the rhubarb samples from June, 2003; [b] compounds, confirmed by comparison with commercial standards; [c] hydroxyanthraquinones; [d] hydroxynaphthalenes.

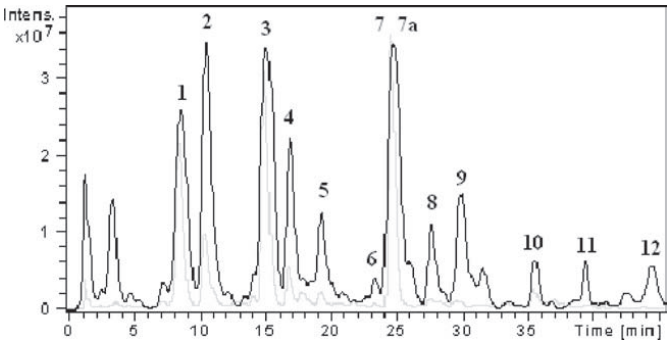


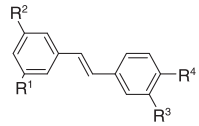
Figure 1. BPC (dark line) and $UV_{306\ nm}$ (light line) chromatograms of *R. rhaponticum* root methanolic extracts. Peaks represent the following identified major stilbenes or anthraquinones or torachrysones: (1) piceatannol glucoside-1; (2) piceatannol glucoside-2 + resveratrol glucoside-1; (3) piceatannol glucoside-3; (4) rhapontin-1; (5) resveratrol galloylglucoside; (6) rhapontigenin galloylglucoside; (7) deoxyrhapontin; (7a) torachrysone glucoside; (8) emodin glucoside + chrysophanol glucoside; (9) deoxyrhapontigenin galloylglucoside; (10) resveratrol dimer-1; (11) resveratrol dimer-2; (12) emodin.

Table 2. Flavonols, identified in the petioles of the *R. rhaponticum*

Flavonoids	t_R, min	$[M-H]^-$	*m/z* of the main daughter ions (in parentheses the relative intensities—intensity of the first, most abundant ion is taken as 100)	Height of EIC peak (mAU)
Myricetin-*O*-rhamnoside	12.2	463	**316**; 317 (56); 271 (6); 179 (4); 287 (2); 151 (1); 301 (1)	686
Quercetin-*O*-rutinoside (rutin)	13.3	609	**301**; 271 (9); 255 (7); 179 (3); 343 (3); 215 (2); 373 (1)	2146
Quercetin-*O*-glucuronide	13.5	477	**301**; 313 (38); 179 (3); 151 (3); 169 (2); 273 (0.5); 431 (0.4)	677
Quercetin-*O*-glucoside	13.8	463	**301**; 179 (3.5); 271 (3); 151 (2.5); 316 (2.5); 255 (1.5); 343 (1)	262
Quercetin-*O*-rhamnoside	16.7	447	**301**; 179 (2.4); 151 (2); 255 (1.5); 271 (1.5); 284 (0.7); 285 (0.6)	1018
Quercetin	23.0	301	**179**; 151 (73); 257 (12); 273 (9); 229 (7); 193 (4); 121 (4); 107 (4)	364

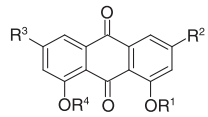
***O*-glycosides.** It is assumed that all of the *O*-hexosides, characterised by an MS^2 constant neutral loss of 162 amu, in *R. rhaponticum* actually contain glucose as the glycosidic group. The identification method used is generally not capable of determining the exact position of glycosidic groups in the aglycone molecule. Comparing the retention times (t_R) of two resveratrol glucosides with the t_R values of commercial *trans*-piceid allowed the identification of resveratrol *O*-glucoside 2 as *trans*-piceid (t_R = 11.4 min; Table 1). Another, more hydrophilic resveratrol glucoside 1 (t_R = 8.6 min) was tentatively identified as resveratroloside (3,5,4′-trihydroxystilbene 4′-*O*-β-D-glucopyranoside). The situation with two piceatannol glucosides is more confusing. Comparing the t_R values of piceatannol glucosides with that of the *trans*-astringin standard (t_R = 8.3 min) enabled piceatannol glucoside 2 to be identified as astringin (3,5,3′,4′-tetrahydroxystilbene-3-*O*-β-D-glucopyranoside). Moreover, the structural analogy with two resveratrol glucosides and theoretical considerations concerning the relative hydrophilicities of the glucoside isomers suggested that piceatannol-glucoside 3, with t_R = 10.6, was a less hydrophilic astringin isomer, with the glycosidic group in a *meta*-position to the –CH=CH– moiety. Obviously, an additional study is needed to specify these piceatannol glucoside isomers. Rhapontins (rhaponticins) and deoxyrhapontin were identified by comparison of their MS^2 spectra with the respective spectra of the commercial standards. The chromatogram of the commercial standard of rhapontin contained two peaks with very similar MS^2 spectra (Table 1). Glycosides of the hydroxyanthraquinones were identified by comparing their MS^2 spectra with those of the respective aglycones and in the case of emodin and rhein glycosides, also with their respective spectra as recently published by Ye *et al*. (2007). In the case of chrysophanol *O*-glucoside, the spectrum contained the daughter ions expected for chrysophanol, including *m/z* = 225 and 235, resulting from the loss of CO or H_2O from the parent ion, respectively (Ye *et al*., 2007). Torachrysone *O*-glucoside was identified by comparing its MS^2 spectrum with the spectrum published by Ye with co-authors (Ye *et al*., 2007).

***O*-acetylglucosides.** *O*-acetylglucosides were identified by their MS^2 spectra, considering that these spectra should contain negative molecular ions of the specific aglycone $[M-H]^-$, the aglycone-*O*-glucoside $[M+162-H]^-$ and at least some fragments characteristic of the respective aglycone. Torachrysone *O*-acetylglucoside was identified by comparing its MS^2 spectrum with the spectrum published by Ye *et al*. (2007). Pterostilbene-*O*-acetylglucosides with pseudomolecular ions at *m/z* = 459 were identified by their MS^2



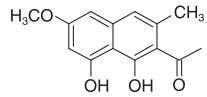
Stilbenes:

Stilbene	R_1	R_2	R_3	R_4
Piceatannol	OH	OH	OH	OH
Resveratrol	OH	OH	H	OH
Rhapontigenin	OH	OH	OH	OCH_3
Isorhapontigenin	OH	OH	OCH_3	OH
Desoxyrhapontigenin	OH	OH	H	OCH_3
Pterostilbene	OCH_3	OCH_3	H	OH



Anthraquinones:

Anthraquinone	R_1	R_2	R_3	R_4
Aloe-emodin	H	CH_2OH	H	H
Chrysophanol	H	CH_3	H	H
Emodin	H	CH_3	OH	H
Physcion	H	CH_3	OCH_3	H
Rhein	H	COOH	H	H



Torachrysone (a naphthalene derivative)

Figure 2. Structure of the identified polyphenolic aglycons.

daughter ions: *m/z* at 255 (ion of the pterostilbene aglycone), at 227 = 255 – 28 (pterostilbene depleted of two methyl groups, at 209 = 255 – 46 (aglycone depleted of two methyl and one hydroxy-group) and at 181 = 255 – 74 (aglycone without two methoxy and one hydroxyl groups). To the best of our knowledge, there is only one previous paper where an occurrence of *O*-acetylglucosides of the hydroxystilbenes or *O*-anthraquinones in any plant has been reported (Ye *et al.*, 2007).

***O*-galloylglucosides.** *O*-galloylglucosides were identified by their MS^2 spectra, considering that the spectra should contain the ions of aglycone $[M-H]^-$, aglycone-*O*-glucoside $[M+162-H]^-$, galloylglucoside (*m/z* = 331) and gallic acid (*m/z* = 169). Actually, all of the MS^2 spectra suspected to belong to galloylglucosides contained a fragment with *m/z* = 313, which was probably associated with the galloylglucoside depleted of one molecule of water. Earlier, resveratrol *O*-galloylglucoside has been identified in the roots of *R. tanguticum*, *R. palmatum* and *R. officinale* (Komatsu *et al.*, 2006), rhapontigenin *O*-galloylglucosides and chrysophanol *O*-galloylglucosides in *R. undulatum* L. (Matsuda *et al.*, 2000a,b; Kageura *et al.*, 2001) and chrysophanyl *O*-galloylglucoside in six various species of genus *R.* including R. *palmatum* and R. *tanguticum* (Ye *et al.*, 2007).

Resveratrol dehydrodimers. Resveratrol dehydrodimers were identified by comparing the MS^2 fragments of the compounds with $[M-H]^- = 453$ with the respective spectra from literature (Jean-Denis *et al.*, 2006; Gonzáles-Barrio *et al.*, 2006; Püssa *et al.*, 2006). No other oligomers of resveratrol were discovered.

The petioles of the *R. rhaponticum* additionally contained several flavonols, namely derivatives of quercetin and myricetin (Table 2) that were identified by comparing their MS^2 spectra with the respective spectra of the commercial standards of quercetin or myricetin.

The results of the study show that roots of *R. rhaponticum* contain a wide variety of hydroxystilbenes. According to the results of the semiquantitative assay it can be concluded that

R. rhaponticum contains far fewer hydroxyanthraquinones. *R. rhaponticum* deserves more attention as a source of medicinally interesting compounds and their mixtures.

References

Aaviksaar A, Haga M, Kuzina K, Püssa T, Raal A and Tsoupras G. 2003. Hydroxystilbenes in roots of *Rheum rhaponticum*. *Proc Estonian Acad Sci Chem* **52**: 99–107.

Agarwal SK, Singh SS, Verma S and Kumar S. 2000. Antifungal activity of anthraquinone derivatives from *Rheum emodi*. *J Ethnopharmac* **72**: 43–46.

Babu SK, Tivari AK, Srinivas PV, Ali AZ, Raju BC and Rao JM. 2004. Yeast and mammalian α-glucosidase inhibitory constituents from Himalayan rhubarb *Rheum emodi* Wall.ex Meisson. *Bioorg Med Chem Lett* **14**: 3841–3845.

Baur JA and Sinclair DA. 2006. Therapeutic potential of resveratrol: the *in vivo* evidence. *Nat Rev Drug Discov* **5**: 493–506.

Blundstone HAW. 1970. Hydroxycinnamic acid derivatives of *Rheum rhaponticum*. *Phytochemistry* **9**: 1677–1679.

Gonzáles-Barrio R, Beltrán D, Cantos E, Gil MI, Espin JC and Tomás-Barberán A. 2006. Comparison of ozone and UV-C treatments on the post-harvest stilbenoid monomer, dimer and trimer induction in var. 'Superior' white table grapes. *J Agric Food Chem* **54**: 4222–4228.

Jean-Denis JB, Pezet R and Tabacchi R. 2006. Rapid analysis of stilbenes and derivatives from downy-mildew-infected grapevine leaves by liquid-chromatography–atmospheric pressure photoionization mass spectrometry. *J Chromatogr A* **1112**: 263–268.

Kageura T, Matsuda H, Morikawa T, Toguchida I, Harima S, Oda M and Yoshikawa M. 2001. Inhibitors from rhubarb on lipopolysaccharide-induced nitric oxide production in macrophages: Structural requirements of stilbenes for the activity. *Bioorg Med Chem* **9**: 323–327.

Kang JH, Park YH, Choi SW, Yang EK and Lee WJ. 2003. Resveratrol derivatives potently induce apoptosis in human promyelocytic leukemia cells. *Exp Mol Med* **35**: 467–474.

Kashiwada Y, Nonaka G and Nishioka I. 1984. Studies on rhubarb (*Rhei Rhizoma*). Isolation and characterization of stilbenes. *Chem Pharm Bull* **32**: 3501–3517.

Ko SK. 2000. A new stilbene diglycoside from *Rheum undulatum*. *Arch Pharmac Res* **23**: 159–162.

Komatsu K, Nagayama Y, Tanaka K, Ling Y, Basnet P and Meselhy MR. 2006. Development of a high performance liquid chromatographic method for systematic quantitative analysis of chemical constituents of rhubarb. *Chem Pharm Bull* **54**: 941–947.

Li H-L, Wang A-B, Huang Y, Liu D-P, Wei C, Williams M, Zhang C-N, Liu, G, Liu Y-Q, Hao D-L, Hui R-T, Lin M and Liang C-C. 2005. Isorhapontigenin, a new resveratrol analog, attenuates cardiac hypertrophy via blocking signaling transduction pathways. *Free Radical Biol Med* **38**: 243–257.

Matsuda H, Morikawa T, Toguchida I, Harima S and Yoshikawa M. 2000a. Antioxidant constituents from rhubarb: structural requirements of stilbenes for the activity and structures of two anthraquinone glucosides. *Bioorg Med Chem*. **9**: 41–50.

Matsuda H, Kageura T, Morikawa T, Toguchida I, Park J-Y, Harima S and Yoshikawa M. 2000b. Effects of stilbene constituents from rhubarb on nitric oxide production in lipopolysaccharide-activated macrophages. *Bioorg Med Chem* **10**: 323–327.

Möller F, Zierau O, Jandausch A, Rettenberger R, Kaszkin-Bettag M and Vollmer G. 2007. Subtype-specific activation of estrogen receptors by a special extract of *Rheum rhaponticum* (ERr 731®), its aglycones and structurally related compounds in U2OS human osteosarcoma cells. *Phytomedicine* **14**: 716–726.

Okabe H, Matsuo K and Nishioka I. 1973. Studies on rhubarb (*Rhei Rhizoma*). II. Anthraquinone glycosides. *Chem Pharm Bull* **21**: 1254–1260.

Okasaka M, Takaishi Y, Kogure K, Fukuzawa K, Shibata H, Higuti T, Honda G, Ito M, Kodzhimatov OK and Ashurmetov O. 2004. New stilbene derivatives from *Calligonum leucocladum*. *J Nat Prod* **67**: 1044–1046.

Park EK, Choo MK, Yoon HK and Kim DH. 2002. Antithrombotic and antiallergic activities of rhaponticin from Rhei Rhizoma are activated by human intestinal bacteria. *Arch Pharm Res* **25**: 528–533.

Piver B, Berthou F, Dreano Y and Lucas D. 2003. Differential inhibition of human cytochrom P450 enzymes by ε-viniferin, the dimer of resveratrol: comparison with resveratrol and polyphenols from alcoholic beverages. *Life Sci* **73**: 1199–1213.

Püssa T, Floren J, Kuldkepp P and Raal A. 2006. Survey of grapevine *Vitis vinifera* stem polyphenols by liquid chromatography–diode array detection–tandem mass spectrometry. *J Agric Food Chem* **54**: 7488–7494.

Rupprich N, Hildebrand H and Kindl H. 1980. Substrate specificity *in vivo* and *in vitro* in the formation of stilbenes. Biosynthesis of rhaponticin. *Arch Biochem Biophys* **200**: 72–78.

Shikishima Y, Takaishi Y, Honda G, Ito M, Takeda Y, Kodzimatov OK and Ashurmetov O. 2001. Phenylbutanoids and stilbene derivatives of *Rheum maximowiczii*. *Phytochemistry* **56**: 377–381.

Wober J, Möller F, Richter T, Unger C, Weigt C, Jandausch A, Zierau O, Rettenberger R, Kaszkin-Bettag M and Vollmer G. 2007. Activation of estrogen receptor-β by a special extract of *Rheum rhaponticum* (ERr 731®), its aglycones and structurally related compounds. *J Steroid Biochem Mol Biol* **107**: 191–201.

Ye M, Han J, Chen H, Zheng J and Guo D. 2007. Analysis of phenolic compounds in rhubarbs using liquid chromatography coupled with electrospray ionization mass spectrometry. *J Am Soc Mass Spectrom* **18**: 82–91.

II

Raudsepp, P., Kaldmäe, H., Kikas, A., Libek, A.-V., Püssa, T., 2010. NUTRITIONAL QUALITY OF BERRIES AND BIOACTIVE COMPOUNDS IN THE LEAVES OF BLACK CURRANT (*RIBES NIGRUM* L.) CULTIVARS EVALUATED IN ESTONIA. Journal of Berry Research. 1, 53−59. doi: 10.3233/BR-2010-006

Journal of Berry Research 1 (2010) 53–59
DOI:10.3233/BR-2010-006
IOS Press

Nutritional quality of berries and bioactive compounds in the leaves of black currant (*Ribes nigrum* L.) cultivars evaluated in Estonia

Piret Raudsepp[a,*], Hedi Kaldmäe[b], Ave Kikas[b], Asta-Virve Libek[b] and Tõnu Püssa[a]
[a]*Department of Food Hygiene, Estonian University of Life Sciences, Tartu, Estonia*
[b]*Polli Horticultural Research Centre, Viljandimaa, Estonia*

Abstract. Polli Horticultural Research Centre (58°7′N; 25°32′E) in Estonia has focused on selecting cultivars with high productivity and suitable to use in local climatic conditions since 1945. Besides important agronomic characteristics, more attention has been recently paid to fruit quality and content of various bioactive compounds.

The results of a biochemical analysis of 4 prospective black currant selections (10B, 2-96-51, 1-96-16, 4-96-1), 4 new cultivars ('Karri', 'Almo', 'Ats', 'Elo') from our own breeding program and 7 introduced cultivars ('Öjebyn', 'Zagadka', 'Ben Sarek', 'Intercontinental', 'Pamyati Vavilova', 'Titania' and 'Pilenai') are presented.

In addition to the analysis of main biochemical characteristics, the anthocyanin content of the berries was determined using high performance liquid chromatography (HPLC). The total anthocyanin content of the berries varied in a wide range. The highest anthocyanin content was found in the cultivar 'Almo' (212 ± 9 mg/100 g) and the lowest in 'Ben Sarek' (83 ± 24 mg/100 g). The ascorbic acid content varied from 98 mg/100 g with 'Ats' to 209 mg/100 g with elite selection 4-96-1.

The polyphenol composition of the black currant leaves was determined by HPLC, the compounds were identified using polyphenol commercial standards and/or compounds mass spectrometric (MS) characteristics.

Keywords: Black currant, seedlings, fruit quality, leaves, poly phenol composition

1. Introduction

Ascorbic acid and anthocyanins are the most highly valued health beneficial compounds in black currants contributing to the antioxidant capacity of the fruit [13]. Anthocyanins have also been associated with fruit colour and are, therefore, important contributors to the quality of the product, they appear to be very stable and remain active after a prolonged frozen storage, processing into juice, wine and jam [9, 13]. The change of focus to quality and nutritional factors in breeding programs in Europe stems from the retail price reduction due to the overproduction of black currants in Europe in 2003–2005 [1]. The focus on the nutritional aspects of the berries is an important sales argument for promoting black currants for fresh consumption. The processors' main requirements are high levels of ascorbic acid and antioxidant properties together with a low acidity and improved sensory characteristics [2]. The anthocyanin content of 4 prospective black currant selections (10B, 2-96-51, 1-96-16, 4-96-1), 4 new cultivars ('Karri', 'Almo', 'Ats', 'Elo') from our own breeding program and 7 introduced cultivars ('Öjebyn', 'Zagadka', 'Ben

*Corresponding author: P. Raudsepp, Department of Food Hygiene, Kreutzwaldi 58A, Tartu 51014, Estonia. Tel.: +372 7313432; Fax: +372 7313432; E-mail: raudsepp.piret@gmail.com.

Sarek', 'Intercontinental', 'Pamyati Vavilova', 'Titania' and 'Pilenai') were analysed in this study, additionally to the main biochemical characteristics.

The leaves of black currant have been used in folk medicine for their diaphoretic and diuretic properties as well as to relieve rheumatic pain [18]. The pharmacological effect other than anti-inflammatory effect of black currant leaves has not been scientifically proved [3, 4]. Their anti-inflammatory effect may be due to the polyphenol compounds that are originally synthesised by plants to protect themselves from pathogens [5]. Surveys have indicated that some of the polyphenol compounds may be exposed on the cuticle of leaves to give a quick response to the microbial attack [25]. Although black currant leaves are widely in use as tea material [17], there are not many scientific studies about the composition of the chemical compounds responsible for the health beneficial effect. We used a mixture of known polyphenol standard compounds, previously known to be present in the leaves of black currant [17, 18] and some additional standards that have been found to be present in other leaves of pharmacological value [6] to determine the composition of polyphenol compounds in the leaves.

2. Materials and methods

2.1. Materials

The anthocyanidin standards and other chemicals, used in sample preparation or chromatographic separation were all with HPLC purity grade: catechin, kaempherol, quercetin-3-glucoside, myricetin, quercetin-3-galactoside from Sigma-Aldrich Inc.; cyanidin-chloride from Carl Roth 4545.1, Germany; delphinidin-chloride from Carl Roth 4537.1, Germany; formic acid from Fluka, USA; quercitrin, Alfa-Aesar; acetonitril, ethanol 96%, hydrochloric acid 37%, NaOH and methanol were with analytical grade; water, ultra pure made prior to analysis using Milli Q by Millipore Corporation.

The chemicals used for biochemical analysis: potassium ferricyanide (III), sulfuric acid, lead subacetate, potassium iodate, NaOH, 2,6-dichloroindophenol, methylene blue and oxalic acid were of analytical grade.

2.2. Collecting of samples

The research was carried out in 2005–2007 and 2009. 500 g of berry samples per cultivar were collected in the middle of the harvesting season from the cultivar evaluation test plots established in 2000. Four prospective selections (10B, 2-96-51, 1-96-16, 4-96-1) and 4 new cultivars ('Karri', 'Almo', 'Ats', 'Elo') [11] from our own breeding program and 7 introduced cultivars ('Öjebyn', 'Zagadka', 'Ben Sarek', 'Intercontinental', 'Pamyati Vavilova', 'Titania' and 'Pilenai') were analysed in three consecutive years 2005, 2006 and 2007.

Fully developed leaves for the qualitative analysis of polyphenols were collected in August 2009 randomly from 6 different bushes of the same cultivar ('Karri') including equal proportions of leaves from different sides and inner and outer range of the bush.

2.3. Methods

The representative samples were prepared from the field samples, 200 g of berries were homogenized using a kitchen blender and analysed on the same day for sugar, soluble solids and ascorbic acid content. The soluble solids content in the homogenized samples were recorded in a refractometer (Abbe WYA-1S, *Optic Ivymen System*) at 20°C, organic acids were determined by titration with 0.1 NaOH. Ascorbic acid was determined using the modified Tillman's method. Ascorbic acid was titrated with 2,6-dichloroindophenol under acid conditions [16]. The ferricyanide method was used for the sugar content analysis [23]. The results are expressed in mg per 100 g of fresh berries. The average weight of berries was determined by weighing 20 berries per sample.

The samples for the anthocyanin content determination were collected at the same time and frozen at 40°C for further preservation.

The leaves were dried at 50°C for 24 h until air-dry and were ground prior to analysis.

2.4. Sample preparation for HPLC analysis of anthocyanins and leaf polyphenols

For the anthocyanidin analysis, 200 g of fresh frozen berries were homogenized with a Heidolph DIAX 900, 2 g of homogenizate was weighed with an *OHAUS* analytical standard scale into a 50 ml centrifuge tube, 3 parallel samples of each. The samples were extracted for 30 min at room temperature with 20 ml ethanol/water/HCl (70:30:1), after which the samples were shaken on a minishaker IKA MS 1 by *IKA-WORKS* for 30 s twice after 5 min. The tubes were then centrifuged with an Eppendorf centrifuge 5810 R at 3220 g at 20°C for 10 min, the supernatant was removed and the samples were extracted once more with 20 ml ethanol/water/HCl (70:30:1) using the same procedure, and for the third time with 5 ml ethanol/water/HCl (70:30:1). The supernatants were combined and 1 ml of the final solution was filtered through a Spartan 13 mm regenerated cellulose membrane filter with a pore size of 0.2 μm (modified [26] and [24]). For the acid hydrolysis of the samples, the final filtrate was taken to 1 M HCl concentration and heated in the Binder oven at 90°C for 60 min [25].

For the polyphenol analysis of the leaves: 0.1 g of dried leaves were weighed into a 15 ml centrifuge tube, 10 ml of 40% ethanol was added, and 3 parallel samples were made. The samples were extracted at room temperature for 24 h. The extracts were centrifuged at 3220 g at 20°C for 10 min. The supernatant was filtered through *Spartan* 13 mm regenerated cellulose membrane filter with a pore size 0.2 μm

2.5. Chromatographic conditions

For detection and quantification of anthocyanins, *Agilent* 1100 Series LC/MSD Trap-XCT with an ESI interface (m/z interval 50–1000 in positive ion mode) and UV-Vis diode array detector was used. HPLC reversed phase column Zorbax 300SB-C^{18} with dimensions of 2.1 × 150 mm with a particle size of 5 μm was used at 35°C, and the speed of the mobile phase was 0.3 ml min^{-1}. The elution was carried out with 1% formic acid in water (mobile phase A) and acetonitril (solvent B), a multistep mobile phase gradient was used as follows: 0 min 95:5 (A:B), linear gradient until 30 min 70:30 (A:B), by 40 min the ratio of A:B was 10:90 and maintained for 50 min, after what the concentration of mobile phase A was raised again to 95% while mobile phase B was dropped to 5% and the system was re-equilibrated 10 min. For the quantification of the anthocyanins, the peak area at 510 nm was used. The detection limit for anthocyanins was 0.1 μg/ml and the quantification limit 0.2 μg/ml. The calibration curve was constructed using a standard mixture of delphinidin and cyanidin.

The analysis of the polyphenol composition of the leaves was conducted on the same chromatographic system but in the negative ionisation mode. The elution gradient was set as follows: 0 min 90:10 [0.1% formic acid in water (A):acetonitril (B)], the linear gradient until 30 min 70:30 (A:B), by 40 min the ratio of A:B was 10:90 and maintained until 50 min and from 50.1 min the concentration of solvent A was raised again to 90% and the concentration of solvent B dropped to 10% and maintained until 60 min to re-equilibrate the system. The calibration curves were constructed using a standard mixture of catechin, chlorogenic acid, quercetin galactoside, quercetin glucoside, myricetin, quercetin rhamnoside also known as quercitrin, and kaempherol.

3. Results and discussion

3.1. Biochemical and morphological characteristics of fruit

The average fruit weight was 1.25 g. The cultivar 'Öjebyn' had the smallest fruits (0.9 g) and 'Karri' the largest ones 1.7 g.

The ascorbic acid content varied from 98 mg/100 g with 'Ats' to a very high level of 209 mg/100 g with elite selection 4-96-1 with average ascorbic acid content of 132.5 mg/100 g (Table 1). 130 mg has been set as the requirement by some producers to ensure the healthiness of the processed product [12].

The sugar content and sugar acid ratio, that are major factors affecting the sweetness of the taste of the berries, were highest with the cultivar 'Pilenai' (12.6% and 4.7, respectively) and lowest with 'Zagadka' (8.4% and 2.7 respectively). Above-the-average sugar acid ratio (3.7) was recorded for the cultivars 'Pamyati Vavilova' 'Titania' 'Intercontinental' and elite selections 4-96-1 and 2-96-51. These selections both have the cultivar 'Pamyati Vavilova' as

Table 1
The average biochemical and morphological characteristics of berries, measured in 2005 and 2007

Cultivar	Soluble solids in juice (%)	Water (%)	Titratable acids (%)	Sugars (%)	Sugar acid ratio	Ascorbic acid (mg/100g)	Average fruit weight (g)
10 B	17.3	79	2.6	9.8	3.5	138	1.2
1-96-16	17.2	76	2.7	11	4.1	134	1
2-96-51	15.6	83	2.7	10.4	3.9	141	1.2
4-96-1	16.3	79	2.6	10.7	4.2	209	1.2
Almo	17.8	79	2.8	9.5	3.4	112	1.2
Ats	16.9	78	2.4	8.8	3.7	98	1.4
Ben Sarek	16.3	80	3.6	8.7	2.4	115	1.5
Elo	17	79	2.6	9.2	3.6	117	1.2
Intercontinental	18.5	79	2.8	10.4	3.7	132	1.5
Karri	16.9	77	2.4	8.9	3.7	134	1.7
Pamyati Vavilova	16.4	79	2.7	11.9	4.4	164	1.2
Pilenai	16	81	2.7	12.6	4.7	110	1.3
Zagadka	16	78	3.1	8.4	2.7	107	1.2
Titania	16	77	3.1	12.6	4.1	141	1.1
Öjebyn	17.9		2.7	8.7	3.2	133	0.9

one of the crossing parents. Both selections have also high acorbic acid contents (209 mg and 141 mg/100 g) similar to their parental genotype 'Pamyati Vavilova'(ascorbic acid content 164 mg/100 g). Comparison with our earlier research [11] including the 4 new cultivars from our breeding program and cultivar 'Öjebyn'used as the standard cultivar, showed that the sugar content in the current study years was slightly higher than average and might have been influenced by the weather conditions and relatively modest yields (data not shown) in these years. The ascorbic acid levels obtained with the above listed cultivars are consistent with the average results obtained previously and show a similar variation between genotypes.

3.2. Analysis of the anthocyanin content of the berries

Our analysis revealed that anthocyanins are present in form of cyanidin and delphinidin glycosides in black currant berries. The prevailing glycosides are delphinidin glucoside (1), delphinidin 3-O-rutinoside (2), cyanidin-glucoside (3), cyaniding 3-O-rutinoside (4) (Fig. 1). Previous studies have revealed similar results [10, 14, 19]. Some authors have discovered a total of 15 different anthocyanin glycosides in black currant berries [21, 22]. After the acid hydrolysis of the samples, it was revealed that cyanidin was slightly dominant over delphinidin (Table 2) in most of the studied cultivars. It is interesting to remark that at the beginning of the berry ripening in the example of 'Pilenai', the cyanidin was largely prevailing over delphinidin 73:23, but in the fully ripened berries, the ratio 57:43 was closer to equal (Table 2). The highest content of anthocyanins was found in cultivars 'Titania', 'Almo', 'Öjebyn', 'Ats', 'Intercontinental' and 'Elo' from which 'Almo', 'Elo' and 'Ats' are from our own breeding programme. Seedling 1-96-16 from our breeding programme showed also a high content of anthocyanins in three consecutive years (Table 2).

It has to be mentioned that in the second year of the study (2006), the weather conditions were not favourable for the berry production, and the content of anthocyanins in the berries gathered approximately at the same time as in the two other years was lower than in the first (2005) and the third (2007) year. It may be the result of incomplete ripening by that time. Previous studies have revealed that anthocyanin content depends on the ripening stage of the berries, increasing during ripening and reaching the maximum when berries are fully ripened [7, 8, 15]. The anthocyanin content may also be influenced by weather conditions [12]. The overall crop yield of berries was likewise lower in the year 2006.

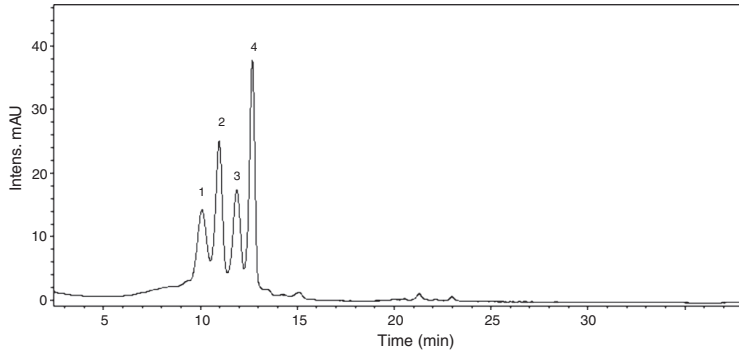


Fig. 1. UV-chromatogram of fruit anthocyanin analysis at 510 nm. 1) delphinidin glucoside, 2) delphinidin 3-O-rutinoside, 3) cyanidin-glucoside, 4) cyaniding 3-O-rutinoside.

Table 2
The average anthocyanin content of black currant berries in 3 consecutive years and the percentage of the main aglycones after acid hydrolysis

	Anthocyanin content of berries (ug/ml)	Delphinidin (%)	Cyanidin (%)
10B	158 ± 21	44	56
1-96-16	221 ± 29	55	45
1-96-51	150 ± 66	41	59
4-96-1	148 ± 33	42	58
Almo	213 ± 9	38	63
Ats	185 ± 65	39	61
Ben Sarek	84 ± 24	37	63
Elo	172 ± 21	40	60
Intercontinental	177 ± 6.5	44	56
Karri	154 ± 89	41	59
Pamjati Vavilova	143 ± 22	40	60
*Pilenai I	0	–	–
*Pilenai II	14 ± 4	27	73
*Pilenai III	111 ± 32	40	60
*Pilenai IV	177 ± 93	43	57
Zagadka	179 ± 43	52	48
Titania	216 ± 29	49	51
Öjebyn	194 ± 52	42	58

*The cultivar 'Pilenai' was measured in four different phases of ripening; stage I being green berries and IV stage the fully ripened stage, collected at the same time as the berries of the other cultivars and seedlings.

3.3. Polyphenol composition of leaves

The results of the HPLC analysis of the leaves are presented in Fig. 2. The analysis was conducted using polyphenol standards (2-catechin, 3-chlorogenic acid, a-quercetin galactoside, 6-quercetin glucoside, b-myricetin, 8-quercitrin,

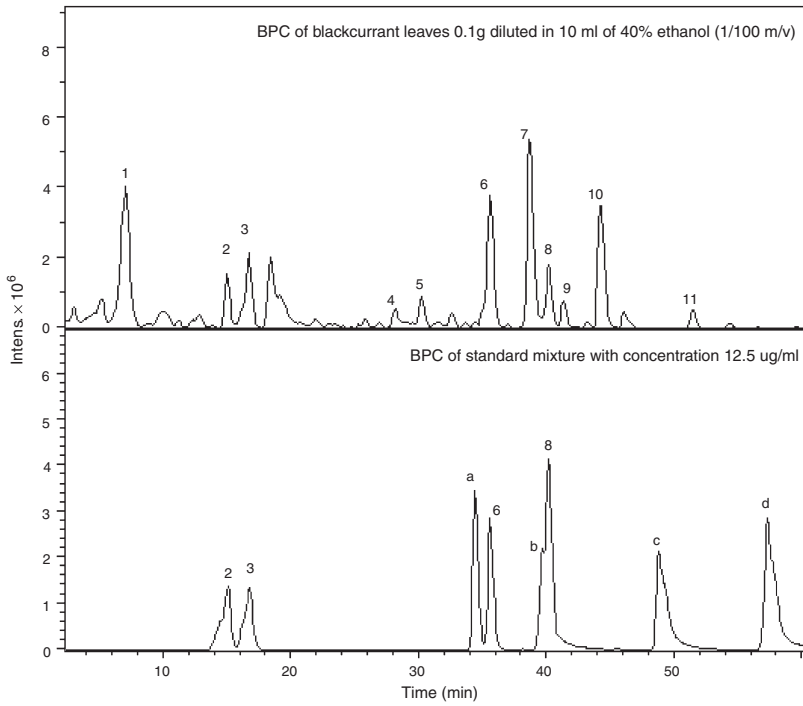


Fig. 2. The base peak chromatogram (BPC) of analysis of the leaves and standard mixture of the known leaf specific polyphenols. The polyphenols found in black currant leaves are marked with numbers repeating the peak numbers on leaf BPC and the standard compounds not found in black currant leaves are marked with letters. 1. gallocatechin; 2. epigallocatechin; 3. chlorogenic acid; 4. unidentified compound with $[M\text{-}H]^- = 383$; 5. myricetin glucoside; 6. quercetin glucoside; 7. quercetin malonylhexoside; 8. mixture of kaempferol glucoside, quercetin rutinoside and quercetin acetylglucoside; 9. mixture of isorhamnetin rutinoside and an unidentified glucoside with $[M\text{-}H]^- = 373$; 10. kaempferol malonylhexoside; 11. isorhamnetin glucoside.

c-quercetin, d-kaempherol) previously known as present in the black currant leaves [17, 18] or in the other leaves of pharmacological value [6]. Some of the standard compounds were present in the black currant leaves as well (peaks 2, 3, 6 and 8). The compounds that were not overlapping with any of the used standards were identified using their molecular weight and MS^2 collision fragments.

The quantitative analysis was performed using the peak area under MS extracted ion chromatograms. The analysis revealed that the concentration of catechin in the dried leaves was 786 mg/100 g; chlorogenic acid 1493 mg/100 g; quercetin glucoside 1947 mg/100 g and quercetin rutinoside 399 mg/100 g. The content of catechin was lower than found in the green tea by Rusak et al. (3330 mg/100 g), but the content of quercetin was significantly higher than found in green tea by Rusak et al. (28 mg/100 g) [20]. The health beneficial effect of black currant leaves compared to the green tea needs to be investigated further.

Acknowledgements

We thank the Estonian Science Foundation (research grants no. 6801 and 7703) and Ministry of Education and Research of Estonia (target financing project no. 1092711s06) for financial support.

References

[1] R.M. Brennan, Currants and gooseberries, in: *Temperate Fruit Crop Breeding*, J.F. Hancock, ed., Springer Science + Business Media B.V., 2008, pp. 177–197.

[2] R.M. Brennan and S.L. Gordon, Future perspectives in black currant breeding, *Acta Hort* **585** (2002), 39–42.

[3] C. Declume, Anti-inflammatory evaluation of hydro alcoholic extract of blackcurrant leaves (Ribes nigrum). *J Ethnopharmacology* **27** (1989), 91–98.

[4] N. Garbacki, M. Kinet, B. Nusgens, D. Desmecht and J. Damas, Proanthocyanidins, from Ribes nigrum leaves, reduce endothelial adhesion molecules ICAM-1 and VCAM-1, *Journal of Inflammation* **9** (2005), 2–9.

[5] R.J. Grayer and T. Kokubun, Plant-fungal interactions: the search for phytoalexins and other antifungal compounds from higher plants, *Phytochemistry* **56** (2001) 253–263.

[6] C.S. Harris, A.J. Burt, A. Saleem, P.M. Le, L.C. Martineau, P.S. Haddad, S.A.L. Benett and J.T. Arnason, A single HPLC-PAD-APCI/MS method for quantitative comparison of phenolic compounds found in leaf, stem, root and fruit extracts of *Vaccinium angustifolium, Phytochemical Analysis* **18** (2007), 161–169.

[7] L. Jaakola, K. Määtä, A.M. Pirttilä, R. Törrönen, S. Kärenlampi and A. Hohtola, Expression of genes involved in anthocyanin biosynthesis in relation to anthocyanin, proanthocyanin and flavonol levels during bilberry fruit development, *Plant Physiology* **130** (2002), 729–739.

[8] W. Kalt and J.E. McDonald, Chemical composition of lowbush blueberry cultivars, *J Amer Soc Hort Sci* **121**(1) (1996), 142–146.

[9] S. Kampuse, K. Kampuss and L. Pizika, Stability of anthocyanins and ascorbic acid in raspberry and blackcurrant cultivars during frozen storage, *Acta Hort (ISHS)* **585** (2002), 507–510.

[10] B. Lapornik, M. Prošek and A. Golc Wondra, Comparison of extracts prepared from plant by-products using different solvents and extraction time, *Journal of Food Engineering* **71** (2005), 214–222.

[11] A. Libek, A. Kikas, H. Kaldmäe and L. Arus, Blackcurrant breeding in Estonia. *Acta Hort (ISHS)* **777** (2008) 77–80.

[12] H.L. Pedersen, Juice quality and yield capacity of black currant cultivars in Denmark, *Acta Hort (ISHS)* **777** (2008), 510–516.

[13] C.E. Lister, P.E. Wilson, K.H. Sutton and S.C. Morrison, Understanding the health benefits of blackcurrants, *Acta Hort (ISHS)* **585** (2002), 443–449.

[14] K. Määttä, A. Kamal-Eldin and R. Törrönen, Phenolic compounds in berries of black, red, green and white currants (*Ribes* sp.), *Antioxidants & Redox Signaling* **3**(6) (2001), 981–993.

[15] R.L. Prior, G. Cao, A. Martin, E. Sofic, J. McEwen, C. O'Brien, N. Lischner, M. Ehlenfeldt, W. Kalt, G. Krewer and C.M. Mainland, Antioxidant capacity as influenced by total phenolic and anthocyanidin content, maturity and variety of *Vaccinium* species, *J Agric Food Chem* **46** (1998), 2686–2693.

[16] B.P. Pleshkov, *Praktikum po biochimiji, Moskva, Kolos*, 1968, pp. 175–176.

[17] Ribis nigri folium, in: *Herbal Drugs and Phytopharmaceuticals*, N.G. Bisset, ed., CRC Press, London, 1994, pp. 421–423.

[18] Ribis nigri folium, in: *E/S/C/O/P Monographs*, ESCOP, London 2003, pp. 425–428.

[19] M. Rubiskiene, I. Jasutiene, P.R. Venskutonis and P. Viskelis, HPLC determination of the composition and stability of blackcurrant anthocyanins, *Journal of Chromatographic Science* **43** (2005), 478–482.

[20] G. Rusak, D. Komes, S. Likić, D. Horžić and M. Kovač, Phenolic content and antioxidative capacity of green and white tea extracts depending on extraction conditions and the solvent used, *Food Chemistry* **110** (2008), 852–858.

[21] J. Scalzo, A. Currie, J. Stephens, T. McGhie and P. Alspach, The anthocyanin composition of different *Vaccinium*, *Ribes* and *Rubus* genotypes, *BioFactors* **34** (2008), 13–21.

[22] R. Slimestad and H. Solheim, Anthocyanins from black currants (*Ribis nigrum* L.), *J Agric Food Chem* **50** (2002), 3228–3231.

[23] V.A. Turkin and E.P. Shirokov, *Tehnologia chranenija i pererabotka plodov i ovoshchei*, *Moskva, Gossudarstvennoje izdatelstvo selsko-hozijaistvennoi literature*, 1960, pp. 149–156.

[24] K. Viljanen, P. Kylli, E.M. Hubbermann, K. Schwarz and M. Heinonen, Anthocyanin antioxidant activity and partition behaviour in whey protein emulsion, *J Agric Food Chem* **53** (2005), 2022–2027.

[25] J. Witzell, R. Gref and T. Näsholm, Plant-part specific and temporal variation in phenolic compounds of boreal bilberry (*Vaccinium myrtillus*) plants, *Biochemical Systematics and Ecology* **31** (2003), 115–127.

[26] X. Wu, L. Gu, R. Prior and S. McKay, Characterization of anthocynins and proanthocyanidins in some cultivars of *Ribes*, *Aronia*, and *Sambucus* and their antioxidant capacity, *J Agric Food Chem* **52** (2004), 7846–7856.

III

Raudsepp, P., Anton, D., Roasto, M., Meremäe, K., Pedastsaar, P., Mäesaar, M., Raal, A., Laikoja, K., Püssa, T., 2013. THE ANTIOXIDATIVE AND ANTIMICROBIAL PROPERTIES OF THE BLUE HONEYSUCKLE (*LONICERA CAERULEA* L.), SIBERIAN RHUBARB (*RHEUM RHAPONTICUM* L.) AND SOME OTHER PLANTS, COMPARED TO ASCORBIC ACID AND SODIUM NITRITE.
Food Control. 31, 129−135.
doi: 10.1016/j.foodcont.2012.10.007

Food Control 31 (2013) 129–135

Contents lists available at SciVerse ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

The antioxidative and antimicrobial properties of the blue honeysuckle (*Lonicera caerulea* L.), Siberian rhubarb (*Rheum rhaponticum* L.) and some other plants, compared to ascorbic acid and sodium nitrite

Piret Raudsepp [a,b,*], Dea Anton [a,b], Mati Roasto [a], Kadrin Meremäe [a], Priit Pedastsaar [a], Mihkel Mäesaar [a,c], Ain Raal [d], Katrin Laikoja [a,e], Tõnu Püssa [a]

[a] Estonian University of Life Sciences, Department of Food Hygiene, Kreutzwaldi 58A, 51014 Tartu, Estonia
[b] Bio-competence Centre of Healthy Dairy Products, Kreutzwaldi 1, 51014 Tartu, Estonia
[c] Veterinary and Food Laboratory, Kreutzwaldi 30, 51006 Tartu, Estonia
[d] University of Tartu, Department of Pharmacy, Nooruse 1, Tartu 50411, Estonia
[e] Estonian University of Life Sciences, Department of Food Science and Technology, Kreutzwaldi 58, 51014 Tartu, Estonia

ARTICLE INFO

Article history:
Received 28 June 2012
Received in revised form
25 September 2012
Accepted 2 October 2012

Keywords:
Lonicera caerulea
Rheum rhaponticum
Antioxidant efficiency
Antibacterial activity

ABSTRACT

The aim of the present study was to evaluate the antioxidative and antimicrobial effects of the ethanol and buffered water infusions of six different plants grown in Estonia, namely Siberian rhubarb (*Rheum rhaponticum* L.), blue honeysuckle (*Lonicera caerulea* L.), tomato (*Lycopersicon esculentum* Mill.), bilberry (*Vaccinium myrtillus* L.), sea-buckthorn (*Hippophae rhamnoides* L.) and black currant (*Ribes nigrum* L.), compared to the food additives ascorbic acid (E300) and sodium nitrite ($NaNO_2$, E250). Additionally, the content of vitamin C and the content of anthocyanins, flavonols and total polyphenols in the studied samples were estimated using High performance liquid chromatography (HPLC) method.

Of the bacterial species used in present study, gram-positive bacteria were represented by *Listeria monocytogenes*, *Kocuria rhizophila* and *Bacillus subtilis*. Gram-negative foodborne pathogenic bacteria were represented by *Escherichia coli* and *Campylobacter jejuni*. Probiotic bacterial species, often used in dairy products, were represented by *Bifidobacterium bifidum* and *Lactobacillus acidophilus*.

The studied plant infusions had both antioxidant and antimicrobial properties. The highest antioxidative effect in the buffered water infusion was found with the berries of blue honeysuckle. However, in the 30% ethanol infusions the antioxidative effect was the highest with the petioles of the Siberian rhubarb, exceeded only by the ascorbic acid solution with the concentration of 10 mg/ml. Among tested plant infusions, the roots of the Siberian rhubarb exhibited the highest antibacterial effect against all bacterial species assayed.

1. Introduction

Food-borne illnesses, the spread of antibiotic-resistant pathogens and concerns regarding safety of synthetic antimicrobial agents have increased consumer demand for the use of plant extracts as natural antimicrobials and antioxidants in foods (Al-Zoreky, 2009; Roasto et al., 2007; Staszewski, Pilosof, & Jagus, 2011; Tiwari et al., 2009). Nature offers many different types of antimicrobial compounds that play an important role in the natural defence of all kinds of living organisms (Rauha et al., 2000; Rodriguez Vaquero, Alberto, & Manca de Nadra, 2007). Plant extracts containing flavonols, other phenolic compounds and organic acids are potent antioxidants and some of them have shown additionally good antimicrobial activity, which makes their possible use in food systems reasonable (Chaovanalikit, Thompson, & Wrolstad, 2004; Choi et al., 2006; Kalogeropoulos, Konteles, Troullidou, Mourtzinos, & Karathanos, 2009).

Probiotic bacteria are widely used in functional foods. Therefore, there is an interest in probiotic bacteria such as *Lactobacillus acidophilus* as well as *Bifidobacteria* together with plant derived compounds, especially in their application in food technology, for their positive impact on human health (Chaovanalikit et al., 2004; Pascual-Teresa, Moreno, & García-Viguera, 2010; Sanders, 2000). Thus, it is important to study the antimicrobial effect of plant

* Corresponding author. Estonian University of Life Sciences, Institute of Veterinary Medicine and Animal Sciences, Department of Food Hygiene, Kreutzwaldi 58A, Tartu 51014, Estonia. Tel.: +372 7 313921.
E-mail address: raudsepp.piret@gmail.com (P. Raudsepp).

0956-7135/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodcont.2012.10.007

derived compounds against probiotic bacteria, whereas applicable plant extracts should not have strong inhibition towards beneficial probiotic bacteria in foods. The plant material used in foods has to be safe also regarding the pharmacological activity. All the plant materials in the present study are the long term consumed components of the human diet, with no concern towards safety when consumed in normal amounts, except of the root of the *Rheum rhaponticum*, which has not been a part of a human diet so far. According to our previous compositional studies (Püssa, Raudsepp, Kuzina, & Raal, 2009), the *R. rhaponticum* root could be an attractive candidate for the use as a natural antioxidant, antimicrobial or functional additive in foods due to its high content of polyphenols. A special extract of the roots of *R. rhaponticum* (ERr 731) has been used as a medication to treat menopausal symptoms since 1993 with the brand name Phytoestrol® since 2006 Phyto-Strol® (Kaszkin-Bettag, Richardson, Rettenberger, & Heger, 2008). The safety studies of the extract of the *R. rhaponticum* root in the concentrations of 100, 300, and 1000 mg of ERr 731/kg body weight (bw)/day in the long term toxicity studies in the beagle dogs of the both sexes have been performed (Kaszkin-Bettag et al., 2008). Additionally, safety studies have been done in perimenopausal women with menopausal symptoms at the concentration of 4 mg of ERr 731 daily (Hasper et al., 2009). However, the additional tests are required to establish the safe doses for the extract or other type of preparations of the root of *R. rhaponticum* in the foods.

Antioxidative efficiency of plant extracts is often measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH), which has deep purple colour and therefore absorbs strongly at 515 nm, whereas reduction product ($DPPH_2$) does not (Tsimogiannis & Oreopoulou, 2004). The loss of colour during the radical reduction reaction is monitored by spectrometric methods. As the reference of antioxidative molecule, ascorbic acid may be used (Floegel, Kim, Chung, Koo, & Chun, 2011). Ascorbic acid was chosen in the current study to be a reference, because it is often used in food industries as an antioxidant in food matrices.

Diffusion methods are widely used to investigate the antibacterial activity of natural substances and plant extracts (Al-Zoreky, 2009; Choi et al., 2006; Rodriguez Vaquero et al., 2007). These assays are commonly based on the use of spots, discs or holes as the reservoir of the solutions of tested substances and generally the holes are preferably used because of capacity (Rauha et al., 2000). Of the bacterial species used in present study, gram-positive bacteria were *Listeria monocytogenes*, *Kocuria rhizophila* and *Bacillus subtilis*. Gram-negative foodborne pathogenic bacteria were represented by *Escherichia coli* and *Campylobacter jejuni*. Gram-positive *Bifidobacterium bifidum* and *L. acidophilus* were selected as probiotic bacterial species often used in dairy products.

In the present study, we report antioxidative and antimicrobial effects of the buffered water and ethanol infusions of the fruits of blue honeysuckle variety 'Roksana' (*Lonicera caerulea* L.); tomato variety 'Vilma' (*Lycopersicon esculentum* Mill.); bilberry (*Vaccinium myrtillus* L.); sea-buckthorn variety 'Avgustinka' (*Hippophae rhamnoides* L.); black currant variety 'Intercontinental'(*Ribes nigrum* L.); the roots and the petioles of Siberian rhubarb (*R. rhaponticum* L.), compared to the antioxidative food additive ascorbic acid (E300) and to the antimicrobial food additive sodium nitrite ($NaNO_2$, E250), respectively. The overall preparation of the plant samples was made to imitate the actual state of the consumable food matrix.

2. Materials and methods

2.1. Plant material

The plant materials tested were the fruits of tomato, bilberry, sea buckthorn, black currant, blue honeysuckle; the roots and petioles of the Siberian rhubarb. The plant samples were collected in 2011, by the researchers of the Polli Horticultural Research Centre of Estonian University of Life Sciences from the collection of genetical resources or from nature (bilberry) and refrigerated at −40 °C for short period until the analysis.

2.2. Bacterial species and strains

Both gram-negative and gram-positive foodborne pathogenic bacteria together with well-known probiotics and bacteria commonly used in sensitivity testing were selected in our study. The antimicrobial activity was tested against selected bacteria such as *B. subtilis*, *K. rhizophila* (ATCC 9341), *L. acidophilus* (ATCC 4356), *B. bifidum* (Bb12), *L. monocytogenes* (ATCC 19115), *E. coli* (NCCB 100282) and *C. jejuni* (ATCC 33291). Bacterial strains were obtained from the strain collections of the Estonian Veterinary and Food Laboratory and Department of Food Hygiene of Estonian University of Life Sciences.

2.3. Chemicals and standards

DPPH (2,2-diphenyl-1-picrylhydrazyl) and ascorbic acid were purchased from Sigma, sodium nitrite from Merck, phosphate buffer with pH = 7, was made with di-sodium hydrogen phosphate dihydrate, $HNa_2O_4P{*}2H_2O$ (Fluka) 98%, M = 177 g/mol and potassium dihydrogen phosphate (H_2KO_4P) M = 136.09 g/mol, 99.5% (Merck). Cyanidin chloride, quinic acid, quercetin, quercitrin, chlorogenic acid, epigallocatechin, catechin, kaempferol, myricetin, procyanidin (B1, B2) and quercetin 3-glucoside were purchased from Sigma, Sigma–Aldrich or Fluka. Chloramphenicol was obtained from LAB M. All the used standards were of HPLC grade purity. All the used solvents were of HPLC grade and purchased from Romil (Cambridge, UK) and formic acid of MS grade was from Fluka.

The solutions were made using ultrapure water (EASYpureRF 1051, Barnstead Thermolyne Co. USA).

2.4. Preparation of plant materials and infusions

Grounded and freeze dried plant material, except the petioles of the Siberian rhubarb, which were thermally dried at 45 °C, was further treated by two different methods: A) the decoction method (Bisset & Wichtl, 1994), similar to the home made tea preparation: to the dry material of 500 mg the aqueous phosphate buffer (pH = 7) was added in the ratio 1:10 (w/v) and the mixture was heated at 95 °C for 10 min. The obtained infusion was cooled down, centrifuged at 3220 *g* for 10 min on the Eppendorf 510R cooling centrifuge (Hamburg, Germany). The obtained supernatant was centrifuged once more and diluted for the measurements up to the ratio 1:80 (w/v). The second preparation method B) dry plant material was macerated in 10 fold excess (w/v) of 30% ethanol at the room temperature for 24 h with periodical shaking (18 r/min) on a rotating shaker (Multi RS-60, BIOSAN, Latvia). The obtained infusion was centrifuged at 3220 *g* on the Eppendorf 510R cooling centrifuge (Hamburg, Germany). The obtained supernatant was centrifuged once more and diluted for the measurements up to 1:80 (w/v).

For HPLC analysis 1:20 (w/v) dilutions, for antioxidant efficiency 1:40 (w/v) dilutions and for antimicrobial activity analyses 1:20, 1:40 and 1:80 (w/v) dilutions together with the initially obtained infusion (A) or infusion (B) were used.

2.5. The free radical scavenging activity

The free radical scavenging activity was determined using the stable free radical DPPH decolourization assay at an absorption

maximum 515 nm using AnalyticJena Specord 200 spectrophotometer (AnalyticJena AG, Germany) with WinASPECT Software package. DPPH methanol solution (6.02×10^{-5} M) was made and kept covered from light in a refrigerator.

Hundred microlitres of tested infusion (A) or infusion (B) was mixed with 3900 μl DPPH solution in a spectrophotometric cuvette and the absorbance was recorded immediately after mixing and after every 10 min during 60 min period until a steady state of the reaction was registered. The reference cuvette (blank), contained aqueous phosphate buffer or 30% ethanol, respectively. The used method was a modification of the methods previously described by Huang, Ou, and Prior (2005) and Helmja, Vaher, Püssa, Raudsepp, and Kaljurand (2008). All the antioxidative efficiency assays were performed in duplicate and as a reference of an antioxidant ascorbic acid in buffered water or in 30% ethanol was used in the concentration of 1 mg/ml and 10 mg/ml on both occasions.

2.6. LC–MS/MS analysis

Samples were analysed using liquid chromatography–electrospray ionization tandem mass spectrometry (LC–DAD–ESI/MS^2) in the negative ion mode on an 1100 Series LC/MSD Trap-XCT (Agilent Technologies, Santa Cruz, CA, USA). The ion trap was connected to an Agilent 1100 Series HPLC instrument consisting of an autosampler, solvent membrane degasser, binary pump and column thermostat. The HPLC 2D ChemStation software with a ChemStation Spectral SW module was used both for process guidance and processing the results. The samples were separated on a Zorbax 300SB-C18 column (2.1 × 150 mm; 5 μm particle size, Agilent Technologies). The column was eluted at 0.3 ml/min with a stepwise mobile phase gradient of 0.1% formic acid (solvent 1) and acetonitrile (solvent 2) at 35 °C. The injection volume of the analysed samples was 5 μl (Mainla, Moor, Karp, & Püssa, 2011). To calculate total vitamin C concentration, ascorbic acid ($[M-H]^-$ = 175) and the dehydroascorbic acid ($[M-H]^-$ = 173) concentrations were summarized and quantified using the ascorbic acid calibration curve, obtained in the same conditions and analysed immediately after preparation of the solutions.

To quantify anthocyanins, a calibration curve of cyanidin chloride was used and the UV chromatogram areas at 520 nm were integrated. To quantify the total polyphenols in the analysed samples, the UV chromatogram areas at 280 nm were integrated and the calibration curve of the mixture containing 10 standard substances (quinic acid, quercitin, quercitrin, chlorogenic acid, epigallocatechin, catechin, kaempferol, myricetin, procyanidin [B_1, B_2], quercetin-3-glucoside), all in concentration of 100, 50, 25, and 12.5 mg/ml was used as reference at the same wavelength. To quantify flavonols in the analysed samples the UV chromatograms at 370 nm were integrated (Table 1).

2.7. Antimicrobial activity test

Slightly modified agar well-diffusion method similar to Al-Zoreky (2009), Kalogeropoulos et al. (2009) and Rodriguez Vaquero et al. (2007) was used. To obtain freshly grown cultures each bacterial species was grown in an appropriate agar medium at the optimal growing conditions. Before experimental use, cultures from the solid medium were subcultivated in liquid media. For *L. monocytogenes*, *E. coli*, *C. jejuni*, *B. bifidum*, *L. acidophilus* 1 μl loopful of bacterial mass was subcultivated in 10 ml of Mueller-Hinton broth (Oxoid) for *L. monocytogenes*, *E. coli*, *C. jejuni* or MRS broth (Oxoid) for *L. acidophilus*, *B. bifidum* and by following incubated at 37 °C for 20 h. Certain amount of incubated bacterial suspension was mixed with 400 ml sterilized 45 °C Mueller-Hinton agar (Oxoid) to obtain final density of 10^6 cfu/ml and then poured into Petri dishes for the solidification at the room temperature. Test-agar pH 7 (Merck) and Test-agar pH 8 (Merck) was used for testing *B. subtilis* and *K. rhizophila*, respectively. The control of the purity of the bacterial suspensions was carried out and the density of the bacterial suspensions was controlled. Wells were made into agar gel (6 mm in diameter) using sterilized stainless steel borer and finally filled with 30 μl of six plant infusions with different dilutions such as 1:10, 1:20, 1:40, 1:80 (w/v). Most of the plates were incubated at 37 °C with the exception of *C. jejuni*, *B. subtilis* and *Kocuria luteus*. *C. jejuni* was incubated at 42 °C, *B. subtilis* and *K. rhizophila* at 30 °C. 1000 mg/l chloramphenicol (LAB M) was used as a positive control (Control (+), Table 2). Ethanol and phosphate buffer (pH = 7) were used as negative controls (Control (−), Table 2), respectively. After 24 h of incubation, the radius of the clear inhibition zone from the edge of the agar well was measured using a ruler to an accuracy of 0.5 mm and the antibacterial effect was calculated as a mean of duplicate tests. A total of 392 ethanol and water infusions of the six plants were prepared 1:10, 1:20, 1:40 and 1:80 (w/v), respectively and analysed for antimicrobial activity against seven different bacterial species (Table 2).

2.8. Statistical analyses

Principal component analysis (PCA), Spearman's rank correlation and *t*-tests were carried out using R statistical software (version 2.14.1). Calibration curves of pure standards were done using linear regression method in Microsoft Excel.

3. Results and discussion

3.1. Antioxidative effect

All the studied plant infusions, except the infusion of the root of Siberian rhubarb, showed higher antioxidant capacity than the

Table 1
Antioxidant efficiency of the studied samples, at the dilution of 1:40 (w/v), together with the content of vitamin C, total anthocyanins, total polyphenols and total flavonols.

Test material	AOX (%) A[a]	AOX (%) B[b]	Vitamin C (mg/ml) A	Vitamin C (mg/ml) B	Anthocyanins (mg/ml) A	Anthocyanins (mg/ml) B	Flavonols (mg/ml) A	Flavonols (mg/ml) B	Polyphenols (mg/ml) A	Polyphenols (mg/ml) B
Lycopersicon esculentum Mill.	37	49	0.02	0.03	0.00	0.00	0.05	0.05	0.5	0.3
Vaccinium myrtillus L.	37	91	0.07	0.15	0.20	0.18	0.24	0.28	1.3	1.7
Hippophae rhamnoides L.	74	74	0.13	0.32	0.00	0.00	0.10	0.11	0.2	0.1
Ribes nigrum L.	84	95	0.12	0.21	0.13	0.11	0.16	0.18	0.8	0.7
Rheum rhaponticum L. root	21	87	0.02	0.03	0.00	0.00	0.26	0.17	7.7	8.5
Rheum rhaponticum L. petiole	48	98	0.10	0.13	0.01	0.01	0.10	0.13	0.6	0.9
Lonicera caerulea L.	86	84	0.14	0.19	0.35	0.36	0.42	0.49	2.4	2.4
Ascorbic acid 1 mg/ml	25	77	1.00	1.00	0.00	0.00	0.00	0.00	0.0	0.0

AOX% – antioxidative efficiency, showing the reduced amount of DPPH.
[a] Infusion, made into phosphate buffer and heated at 95 °C for 10 min.
[b] Infusion, obtained from infusing plant material in 30% ethanol for 24 h at room temperature, with continuous shaking.

Table 2
Antimicrobial activity[a] of studied plant water (A) and ethanol (B) infusions against selected bacteria.

Test material	Conc. (w/v)	*B. subtilis*		*K. rhizophila*		*L. monocytogenes*		*E. coli*		*B. bifidum*		*L. acidophilus*		*C. jejuni*	
		A	B	A	B	A	B	A	B	A	B	A	B	A	B
L. esculentum Mill.	1:10	–	–	–	–	–	–	–	–	–	–	–	2	–	–
	1:20	–	–	–	–	–	–	–	–	–	–	–	1	–	–
	1:40	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	1:80	–	–	–	–	–	–	–	–	–	–	–	–	–	–
V. myrtillus. L	1:10	–	–	1	1.5 ± 0.7	0.5	–	1	0.5	–	–	–	3	1	1
	1:20	–	–	–	0.5	–	–	–	–	–	–	–	2	0.5	0.5
	1:40	–	–	–	–	–	–	–	–	–	–	–	1	–	–
	1:80	–	–	–	–	–	–	–	–	–	–	–	–	–	–
H. rhamnoides L.	1:10	1.5 ± 0.7	3	2	1.5 ± 0.7	–	–	–	2	–	–	–	7.5 ± 0.7	–	–
	1:20	–	1.5 ± 0.7	–	0.5	–	–	–	1	–	–	–	5	–	–
	1:40	–	0.5	–	–	–	–	–	0.5	–	–	–	2.5 ± 0.7	–	–
	1:80		–	–	–	–	–	–	–	–	–	–	0.5	–	–
R. nigrum L.	1:10	1	1.5 ± 0.7	3	1.5 ± 0.7	–	–	1	1.5	0.5	–	–	1	0.5	0.5
	1:20	0.5	0.75 ± 0.7	1	0.75 ± 0.35	–	–	–	1	–	–	–	–	–	–
	1:40	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	1:80	–	–	–		–	–	–	–	–	–	–	–	–	–
R. rhaponticum L. root	1:10	9	4	3	4	1	3	1	1	1	2.5 ± 0.7	–	4.5 ± 0.7	10	10
	1:20	6	3	2	3	–	2	–	0.5	–	1.5 ± 0.7	–	3.5 ± 0.7	4	4 ± 1.4
	1:40	1.5 ± 0.7	2	1	2	–	1	–	–	–	0.75 ± 0.35	–	2.5 ± 0.7	–	–
	1:80	0.5	0.5	–	1	–	0.5	–	–	–	–	–	1.75 ± 0.35	–	–
R. rhaponticum L. petiole	1:10	–	–	2	3.5 ± 0.7	–	0.75 ± 0.35	0.75 ± 0.35	0.75 ± 0.35	–	–	3.5 ± 0.7	4	–	–
	1:20	–	–	1	2	–	0.5	0.5	0.5	–	–	2	3	–	–
	1:40	–	–	–	1	–	–	–	–	–	–	1	2	–	–
	1:80	–	–	–	–	–	–	–	–	–	–	–	–	–	–
L. caerulea L.	1:10	2	2.5 ± 0.7	2	3	1	1	–	2	–	–	–	1.5 ± 0.7	2	1
	1:20	0.5	1	–	0.5	–	–	–	1	–	–	–	–	1	0.5
	1:40	–	1	–	–	–	–	–	–	–	–	–	–	–	–
	1:80	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Control (+)		15	14	12	9	5	7	4	8	5	4	8	9	14	14
Control (−)		–	–	–	–	–	–	–	–	–	–	–	–	–	–

– no visible growth inhibition detected.
A – infusion, made into phosphate buffer and heated at 95 °C for 10 min.
B – infusion, obtained from infusing plant material in 30% ethanol for 24 h at room temperature, with continuous shaking.
[a] Values are mean of the inhibition zones ± SD (standard deviation) obtained from analyses in duplicate, zero SD values are not shown.

solution of ascorbic acid with the concentration of 1 mg/ml, prepared in the buffered water (A). The content of ascorbic acid in all plant infusions (A) and 30% ethanol infusions (B) was measured. It was found that sample heating prior to analysis resulted in remarkably lower ascorbic acid content compared to the solutions, prepared in 30% ethanol at the room temperature (Table 1). This is in accordance with the results of the previous studies, indicating high susceptibility of ascorbic acid to the temperature rise (Zhang & Hamauzu, 2004). It may be one of the reasons why the antioxidative properties of the buffered water infusions were weaker than in the ethanol infusions (Table 1). The other reason could be that ethanol infusions had better antioxidant properties due to the antioxidative effect of the lipophilic compounds present in ethanol solutions but not in the water infusions. In the plant material the antioxidative properties may depend on the presence of the water soluble ascorbic acid as well as the semi polar polyphenolic compounds or unpolar compounds, such as carotenoids (Zhang & Hamauzu, 2004). Regarding antioxidative properties, the order of the studied plant infusions in buffered water (A), starting from the highest antioxidative effect, was: blue honeysuckle, black currant, ascorbic acid (10 mg/ml), sea buckthorn, the petioles of the Siberian rhubarb, bilberry/tomato, ascorbic acid (1 mg/ml) and roots of the Siberian rhubarb (Table 1). Bilberry had the same antioxidative properties as tomato. Above mentioned results are applicable to the heated (buffered) water solutions, where the unpolar compounds of the studied plant matrices, which may also have antioxidative properties, have not diffused into the solution. In 30% ethanol infusion (B), however, the order of antioxidative properties of the studied plant materials was different. Starting with the highest antioxidative effect the antioxidativities decreased in the order: ascorbic acid (10 mg/ml), rhubarb petiole, black currant, bilberry, rhubarb root, blue honeysuckle, ascorbic acid (1 mg/ml), sea buckthorn, tomato (Table 1). The antioxidative effect of the blue-coloured berries was rather difficult to estimate as the DPPH absorbs light at the same wavelength (515 nm) as the anthocyanins of the berries (Chaovanalikit et al., 2004). More accurate method to estimate the antioxidative effect of the blue-coloured berries could be the ABTS assay where the oxidation reaction is monitored at 734 nm (Floegel et al., 2011) or HPLC/MS^2 method, by measuring the loss of DPPH by its molecular weight. Nevertheless, at the dilution rate of 1:40 (w/v) the tested plant material did not interfere with the colour loss of DPPH during the reaction, the results of our study are presented in Table 1. Out of the studied plant parts, the berries of the blue honeysuckle displayed the highest antioxidant properties in the water infusion, whereas in ethanol infusion the best antioxidant properties were found for rhubarb petiole, followed by black currant, bilberry and the blue honeysuckle. The results showed that antioxidant properties of the plants depended on the solvent. The antioxidant activity was positively correlated with the ascorbic acid content in the water infusion ($r(s) = 0.93$, $p < 0.001$), but no statistically significant correlations were found between the studied parameters in the ethanol infusion. Previous studies have reported that antioxidative properties of the plants are dependent on the content of the polyphenols (Villaño, Fernández-Pachon, Moyá, Troncoso, & García-Parrilla, 2007), especially on the anthocyanins (Chaovanalikit et al., 2004; Paśko et al., 2009; Viljanen, Kylli, Hubbermann, Schwarz, & Heinonen, 2005). The highest content of anthocyanins in the current study was found in the blue honeysuckle. The profile of anthocyanins in the blue honeysuckle is well characterized in the study of Chaovanalikit et al. (2004). The highest content of polyphenols in our study was found in the Siberian rhubarb root, which regardless, had the lowest antioxidative capacity in the water infusion. The result could be explained by the finding, that main constituents of the Siberian rhubarb root infusion are hydroxystilbenes (Püssa et al., 2009), mostly characterized by modest antioxidativities (Helmja et al., 2008), and also by the possible matrix–solvent interactions (Tsimogiannis & Oreopoulou, 2004). At the same time, the berries with higher anthocyanin content had also higher antioxidative properties, which are in accordance with the previous studies of Paśko et al. (2009), and Viljanen et al. (2005).

3.2. Antimicrobial effect

Our findings confirmed the results of the study by Sebiomo, Awofodu, Awosanya, Awotona, and Ajayi (2011) that ethanol infusions have higher antibacterial effect than the water infusions. Among the seven tested microorganisms, *L. acidophilus*, *K. rhizophila*, *E. coli* and *B. subtilis* were more susceptible to the plant ethanol infusions, whereas *B. bifidum*, *L. monocytogenes* and *C. jejuni* were less susceptible. Plant water infusions had strongest growth inhibition effect against *K. rhizophila*, *B. subtilis* and *C. jejuni*, whereas *L. monocytogenes*, *B. bifidum*, *L. acidophilus* and *E. coli* were less sensitive. In our study, Siberian rhubarb root, blue honeysuckle and black currant water infusions were most effective against both gram-positive bacteria like *B. subtilis* and *K. rhizophila* as well as against gram-negative bacteria such as *C. jejuni*, and the inhibition zones varied between 0.5 and 10 mm, being bigger at the highest concentration of the plant materials 1:10 (w/v) (Table 2).

Our study showed that some plant infusions, e.g. of blue honeysuckle and Siberian rhubarb petiole can elicit high antibacterial activity against food-borne bacteria such as *L. monocytogenes*, *E. coli* or *C. jejuni* without strong inhibition towards probiotics, especially *B. bifidum*. Therefore, selected plant infusions in combination with the tested probiotic bacteria can be used in food processing as potential antioxidants, antimicrobials, and functional ingredients (Table 2).

Kosikowska, Smolarz, and Malm (2010) have found that all of the *Rheum* spp. (rhubarb) infusions had higher antimicrobial activity against strains of gram-positive bacteria (*Staphylococcus* spp.) than against those of gram-negative bacteria (*E. coli*). In our study it was shown that Siberian rhubarb root water and ethanol infusions had the strongest effect towards both gram-negative (*C. jejuni*) and gram-positive bacteria (*B. subtilis*). *L. monocytogenes* is one of the most important gram-positive bacteria causing food production problems; therefore it was chosen for our susceptibility study. In a recent study by Xi, Sullivan, Jackson, Zhou, and Sebranek (2012), it was found that 3% cranberry powder in naturally-cured frankfurter sausages significantly reduced *L. monocytogenes* growth and could be used instead of sodium nitrite in natural and organic processed meats. In our study *L. monocytogenes* was found not to be susceptible to most of the tested plant infusions with the exception of the blue honeysuckle and root/petiole of Siberian rhubarb. Additionally, *E. coli* and *C. jejuni* were selected in our study. *E. coli* is a well-known component of human gut microbiota, and some enterohemorrhagic strains of *E. coli* have recently caused serious food-borne poisoning cases in the European Union (EU). Among the studied plants, the blue honeysuckle and sea buckthorn ethanol infusions had the highest antibacterial activity against *E. coli*. The strong or moderate antimicrobial activity was measured for Siberian rhubarb root, blue honeysuckle and black currant infusions. *Campylobacter* spp. is the most important gastroenteritis causing bacteria in EU associated mainly with the consumption of contaminated chicken meat (Meremäe et al., 2010). In our study it was found that *C. jejuni* was the most susceptible bacterium, showing the widest inhibition zones with rhubarb root, whereas the same results were reported both for water and ethanol infusions with inhibition zones from 4 to 10 mm for the dilutions of 1:20 and 1:10 (w/v) (Table 2). The strong antibacterial effect of

Siberian rhubarb root infusions should be further studied with respect to broiler chicken meat which is very often contaminated with *Campylobacter* spp.

There was very limited antibacterial effect of tomato infusions in our study. Contrary, Taveira et al. (2010) showed that tomato seeds in different extracts had effect against gram-positive gastrointestinal bacteria but all tested gram-negative bacteria were resistant to different extracts which was explained by different structure of cell wall of these bacteria. We did not study tomato seeds separately that is one of the possible explanations to the differences compared to the results of Taveira et al. (2010).

There were no clear differences on susceptibility patterns of gram-negative and gram-positive bacteria in our study.

Sodium nitrite is used in cured meats because of colour, flavour, antioxidant effects and as an effective antimicrobial agent to control the growth of certain food-borne pathogenic bacteria, especially *Clostridium botulinum* (Shahidi & Pegg, 1992). Additionally, it is known that by far not all bacteria are inhibited by $NaNO_2$ and for some of food-borne pathogenic bacteria; sodium nitrite only slows the bacterial growth (Xi, Sullivan, Jackson, Zhou, & Sebranek, 2011). In our study sodium nitrite 2.5% and 5% buffered water solutions did not show any antimicrobial activity against tested bacteria even at different pH ranges from 4.0 to 6.5. Our results may be explained by bacterial species selection or by the use of agar-well method instead of nutrient broth media which probably can more properly imitate the action of nitrite in acidified food products. Generally, the antibacterial effect of tested plants was dependent on the solvent (A or B), dilution rates (1:10, 1:20, 1:40 or 1:80) and tested bacterial strains. Compared to plant water infusions, the antimicrobial properties of ethanol infusions of bilberry, sea buckthorn, black currant, Siberian rhubarb and blue honeysuckle were more effective against tested bacteria. Compared to other dilutions, the strongest dilution of 1:10 both for water and ethanol infusions were found to be the most effective.

Among all the tested plant ethanol and water infusions, the root of the Siberian rhubarb showed the best antimicrobial properties against tested bacteria. The rhubarb root indicated antimicrobial activity against *B. subtilis*, *K. rhizophila*, *L. monocytogenes*, *E. coli*, *L. acidophilus*, *B. bifidum* and *C. jejuni* with the exception of rhubarb root water infusion which had no antimicrobial activity against *L. acidophilus*. The maximum inhibitory zone was determined for *C. jejuni* (10 mm of inhibition zone) followed by *B. subtilis* (4–9 mm) at the dilution of 1:10 (w/v). The smallest measured inhibition zones (0.5 mm) were detected against *B. subtilis* and *L. monocytogenes* at the dilution of 1:80 (w/v). Compared to the root of the Siberian rhubarb, the Siberian rhubarb petiole water and ethanol infusions showed antibacterial activity in lesser amount, against *K. rhizophila* (inhibition zones 1–3.5 mm), *E. coli* (0.5–0.75 mm) and *L. acidophilus* (2–4 mm) at the dilutions 1:10 and 1:20. Fig. 1 is illustrating the influence of the rhubarb root infusions (1:10, 1:20, 1:40, 1:80) to the growth of *B. subtilis*.

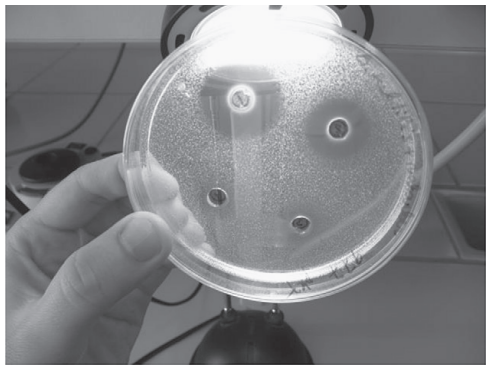


Fig. 1. The influence of the Siberian rhubarb root water infusions (1:10, 1:20, 1:40, 1:80 w/v) to the growth of *B. subtilis*.

4. Conclusions

Among the studied plants, the blue honeysuckle had the highest content of anthocyanins and showed continuously good antioxidative effect in water and ethanol solution as well as the antibacterial activity, whereas in the water infusion the antibacterial activity against probiotic bacteria was not detected, which makes the blue honeysuckle a good functional ingredient candidate to use in probiotic foods. The results showed that antioxidant properties of the plants depended on the used solvent and on the content of vitamin C and anthocyanins.

Among all the tested plant infusions, the ethanol infusion of the root of Siberian rhubarb showed the highest antimicrobial activity against all the tested bacteria including probiotic bacteria in the ethanol infusion. The ethanol infusion of the berries of the sea buckthorn showed also high antimicrobial activity against *L. acidophilus*, therefore it could be used in the probiotic functional foods in the form of water infusion. All the more, the antioxidative properties of the sea buckthorn were similar, both in the water and ethanol infusions. The infusions of tomato and sodium nitrate showed minimum or no antimicrobial activity against tested bacteria. In the light of the results of the current study it can be concluded that the roots and petioles of Siberian rhubarb and the berries of the blue honeysuckle, bilberry, black currant and sea buckthorn may have the potential use in the food industry as natural antioxidants and/or antimicrobials and functional ingredients in foods. However, studies with different food matrices which prove that chemical, physical and sensory attributes are reliable, have yet to be performed.

Acknowledgement

Hedi Kaldmäe and Ave Kikas from Polli Horticultural Research Centre of the Estonian University of Life Sciences are gratefully acknowledged for providing the plant materials for the tests.

The research is co-financed by:

European Community's Regional Development Fund in the Framework of the Competence Centre Programme of the Enterprise Estonia under project No EU 30002 of Bio-Competence Centre of Healthy Dairy Products;

Estonian Research Council (Sihtasutus Eesti Teadusagentuur, ETA-g) Grant No. 9315.

References

Al-Zoreky, N. S. (2009). Animicrobial activity of pomegranate (*Punica granatum* L.) fruit peels. *International Journal of Food Microbiology, 134*, 244–248.

Bisset, N. G., & Wichtl, M. (1994). *Herbal drugs and phytopharmaceuticals: A handbook for practice on scientific basis*. Stuttgard: Medpharm Scientific Publishers.

Chaovanalikit, A., Thompson, M. M., & Wrolstad, R. E. (2004). Characterization and quantification of anthocyanins and polyphenolics in blue honeysuckle (*Lonicera caerulea* L.). *Journal of Agricultural and Food Chemistry, 52*, 848–852.

Choi, Y. M., Noh, D. O., Cho, S. Y., Suh, H. J., Kim, K. M., & Kim, J. M. (2006). Antioxidant and antimicrobial activities of propolis from several regions of Korea. *LWT – Food Science and Technology, 39*, 756–761.

Floegel, A., Kim, D.-O., Chung, S. J., Koo, S. I., & Chun, O. K. (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *Journal of Food Composition and Analysis, 24*, 1043–1048.

Hasper, I., Ventskovskiy, B. M., Rettenberger, R., Heger, P. W., Riley, D. S., & Kaszkin-Bettag, M. (2009). Long-term efficacy and safety of the special extract ERr 731 of

Rheum rhaponticum in perimenopausal women with menopausal symptoms. *Menopause, 16*, 117–131.
Helmja, K., Vaher, M., Püssa, T., Raudsepp, P., & Kaljurand, M. (2008). Evaluation of antioxidative capability of the tomato (*Solanum lycopersicum*) skin constituents by capillary electrophoresis and high-performance liquid chromatography. *Electrophoresis, 29*, 3980–3988.
Huang, D., Ou, B., & Prior, R. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry, 53*, 1841–1856.
Kalogeropoulos, N., Konteles, S. J., Troullidou, E., Mourtzinos, I., & Karathanos, V. T. (2009). Chemical composition, antioxidant activity and antimicrobial properties of propolis extracts from Greece and Cyprus. *Food Chemistry, 116*, 452–461.
Kaszkin-Bettag, M., Richardson, A., Rettenberger, R., & Heger, P. W. (2008). Long-term toxicity studies in dogs support the safety of the special extract Err 731 from the roots of *Rheum rhaponticum*. *Food and Chemical Toxicology, 46*, 1608–1618.
Kosikowska, U., Smolarz, H. D., & Malm, A. (2010). Antimicrobial activity and total content of polyphenols of *Rheum* L. species growing in Poland. *Central European Journal of Biology, 5*, 814–820.
Mainla, L., Moor, U., Karp, K., & Püssa, T. (2011). The effect of genotype and rootstock on polyphenol composition of selected apple cultivars in Estonia. *Žemdirbystė-Agriculture, 98*, 63–70.
Meremäe, K., Elias, P., Tamme, T., Kramarenko, T., Lillenberg, M., Karus, A., et al. (2010). The occurrence of *Campylobacter* spp. in Estonian broiler chicken production in 2002–2007. *Food Control, 21*, 272–275.
Pascual-Teresa, S., Moreno, D. A., & García-Viguera, C. (2010). Flavanols and anthocyanins in cardiovascular health: a review of current evidence. *International Journal of Molecular Sciences, 11*, 1679–1703.
Paśko, P., Bartoń, H., Zagrodzki, P., Gorinstein, S., Folta, M., & Zachwieja, Z. (2009). Anthocyanins, total polyphenols and antioxidant activity in amaranth and quinoa seeds and sprouts during their growth. *Food Chemistry, 115*, 994–998.
Püssa, T., Raudsepp, P., Kuzina, K., & Raal, A. (2009). Polyphenolic composition of roots and stalks of *Rheum rhaponticum* L. *Phytochemical Analysis, 20*, 98–103.
Rauha, J.-P., Remes, S., Heinonen, M., Hopia, A., Kähkönen, M., Kujala, T., et al. (2000). Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *International Journal of Food Microbiology, 56*, 3–12.
Roasto, M., Juhkam, K., Tamme, T., Hörman, A., Häkkinen, L., Reinik, M., et al. (2007). High level of antimicrobial resistance in *Campylobacter jejuni* isolated from broiler chickens in Estonia in 2005 and 2006. *Journal of Food Protection, 70*, 1940–1944.
Rodriguez Vaquero, M. J., Alberto, M. R., & Manca de Nadra, M. C. (2007). Antibacterial effect of phenolic compounds from different wines. *Food Control, 18*, 93–101.
Sanders, M. E. (2000). Considerations for use of probiotic bacteria to modulate human health. *Journal of Nutrition, 130*, 384–390.
Sebiomo, A., Awofodu, A. D., Awosanya, A. O., Awotona, F. E., & Ajayi, A. J. (2011). Comparative studies of antibacterial effect of some antibiotics and ginger (*Zingiber officinale*) on two pathogenic bacteria. *Journal of Microbiology and Antimicrobials, 3*, 18–22.
Shahidi, F., & Pegg, R. B. (1992). Nitrite-free meat curing systems: update and review. *Food Chemistry, 43*, 185–191.
Staszewski, M., Pilosof, A. M. R., & Jagus, R. J. (2011). Antioxidant and antimicrobial performance of different Argentinean green tea varieties as affected by whey proteins. *Food Chemistry, 125*, 186–192.
Taveira, M., Silva, L. R., Vale-Silva, L. A., Pinto, E., Valentao, P., Ferreres, F., et al. (2010). *Lycopersicon esculentum* seeds: an industrial byproduct as an antimicrobial agent. *Journal of Agricultural and Food Chemistry, 58*, 9529–9536.
Tiwari, B. K., Valdramidis, V. P., O'Donell, C. P., Muthukumarappan, K., Bourke, P., & Cullen, P. J. (2009). Application of natural antimicrobials for food preservation. *Journal of Agricultural and Food Chemistry, 57*, 5987–6000.
Tsimogiannis, D. I., & Oreopoulou, V. (2004). Free radical scavenging and antioxidant activity of 5,7,3′,4′-hydroxy-substituted flavonoids. *Innovative Food Science & Emerging Technologies, 5*, 523–528.
Viljanen, K., Kylli, P., Hubbermann, E. M., Schwarz, K., & Heinonen, M. (2005). Anthocyanin antioxidant activity and partition behaviour in whey protein emulsion. *Journal of Agricultural and Food Chemistry, 53*, 2022–2027.
Villaño, D., Fernández-Pachon, M. S., Moyá, M. L., Troncoso, A. M., & García-Parrilla, M. C. (2007). Radical scavenging ability of polyphenolic compounds towards DPPH free radical. *Talanta, 71*, 230–235.
Xi, Y., Sullivan, G. A., Jackson, A. L., Zhou, G. H., & Sebranek, J. G. (2011). Use of natural antimicrobials to improve the control of *Listeria monocytogenes* in a cured cooked meat model system. *Meat Science, 88*, 503–511.
Xi, Y., Sullivan, G. A., Jackson, A. L., Zhou, G. H., & Sebranek, J. G. (2012). Effects of natural antimicrobials on inhibition of *Listeria monocytogenes* and on chemical, physical and sensory attributes of naturally-cured frankfurters. *Journal of Meat Science, 90*, 130–138.
Zhang, D., & Hamauzu, Y. (2004). Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. *Food Chemistry, 88*, 503–509.

IV

Raudsepp, P., Koskar, J., Anton, D., Meremäe, K., Kapp, K., Laurson, P., Bleive, U., Kaldmäe, H., Roasto, M., Püssa, T., 2019. ANTIBACTERIAL AND ANTIOXIDATIVE PROPERTIES OF DIFFERENT PARTS OF GARDEN RHUBARB, BLACKCURRANT, CHOKEBERRY AND BLUE HONEYSUCKLE.
Journal of the Science of Food and Agriculture, 99, 2311−2320.
doi: 10.1002/jsfa.9429

Research Article

Received: 2 February 2018 Revised: 2 July 2018 Accepted article published: 16 October 2018 Published online in Wiley Online Library: 3 December 2018

(wileyonlinelibrary.com) DOI 10.1002/jsfa.9429

Antibacterial and antioxidative properties of different parts of garden rhubarb, blackcurrant, chokeberry and blue honeysuckle

Piret Raudsepp,[a*] Julia Koskar,[a,b] Dea Anton,[a] Kadrin Meremäe,[a] Karmen Kapp,[c] Peeter Laurson,[d,e] Uko Bleive,[e] Hedi Kaldmäe,[e] Mati Roasto[a†] and Tõnu Püssa[a†]

Abstract

BACKGROUND: It is important to find plant materials that can inhibit the growth of *Listeria monocytogenes* and other food-spoiling bacteria both *in vitro* and *in situ*. The aim of the study was to compare antibacterial and antioxidative activity of selected plant–ethanol infusions: leaves and berries of blackcurrant (*Ribes nigrum* L.), berries of chokeberry (*Aronia melanocarpa* (Michx.) Elliott) and blue honeysuckle (*Lonicera caerulea* L. var. *edulis*); petioles and dark and light roots of garden rhubarb (*Rheum rhaponticum* L.) for potential use in food matrices as antibacterial and antioxidative additives.

RESULTS: The strongest bacterial growth inhibition was observed in 96% ethanol infusions of the dark roots of rhubarbs. In 96% ethanol, nine out of ten studied plant infusions had antibacterial effect against *L. monocytogenes*, but in 20% ethanol only the infusions of dark rhubarb roots had a similar effect. Chokeberry and other berries had the highest antioxidative activity, both in 20% and 96% ethanol infusions.

CONCLUSION: The combination of dark rhubarb roots or petioles and berries of black chokeberry, blackcurrant or some other anthocyanin-rich berries would have potential as both antibacterial and antioxidative additives in food.

Keywords: antibacterial activity; antioxidative activity; *Aronia*; *Lonicera*; *Rheum*; *Ribes*

INTRODUCTION

As a result of increasing customer awareness, there is a trend to seek new natural food additives that can be used as antibacterial (AB) and/or antioxidative (AO) agents in foods. It is particularly important to find plant materials that can inhibit the growth of *Listeria monocytogenes*, resistant to many environmental stress factors. In addition, *Campylobacter jejuni* is a highly prevalent bacterium in broiler chicken meat of Baltic origin and the most often reported bacterial cause of human intestinal infections.[1]

The synergistic effect of different polyphenolic compounds is mainly responsible for antimicrobial,[2] antioxidative,[3,4] health beneficial[5–7] and plant protective properties[8] of a plant material. There are studies where polyphenolic composition of rhubarb roots[9], blackcurrant leaves and berries,[10,11] edible honeysuckle berries[12,13] and chokeberry berries[14,15] have been sufficiently described. Kosikowska *et al.*[16] and Raudsepp *et al.*[17] have shown a very strong AB effect of garden rhubarb roots. Hasper *et al.*[18] have established that only minimal toxicity concerns exist regarding the use of garden rhubarb root preparations for human internal consumption.

According to Zheng *et al.*[19] and Vagiri *et al.*,[11] polyphenolic composition of a plant product depends on variety, maturity and part of the plant, weather and processing technology. Raudsepp *et al.*[10] and Vagiri *et al.*[20] have ascertained that European blackcurrant varieties may have two- to threefold differences in the anthocyanin content, even if grown under the same conditions. Differences

* *Correspondence to: P Raudsepp, Department of Food Hygiene and Veterinary Public Health, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Tartu 51006, Estonia, Kreutzwaldi 56/3. E-mail: raudsepp.piret@gmail.com*

† *These authors contributed equally to the work.*

a *Chair of Food Hygiene and Veterinary Public Health, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Tartu, Estonia, Kreutzwaldi 56/3*

b *Department of Food Microbiology, Veterinary and Food Laboratory, Tartu, Estonia*

c *Division of Pharmaceutical Biology, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland*

d *Faculty of Science and Technology, Institute of Chemistry, University of Tartu, Tartu, Estonia*

e *Polli Horticultural Research Centre, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Viljandimaa, Estonia*

Table 1. Media used and incubation conditions for different bacteria

Bacterial culture	Agar media	Incubation conditions
Gram negative		
Campylobacter jejuni ATCC 33291	Columbia blood agar (Oxoid) + 5% lysed horse blood (Oxoid)	42 °C, 48 h, micro aerobic
Salmonella Enteritidis ATCC 13076	Mueller Hinton agar (Oxoid)	37 °C, 48 h, aerobic
Escherichia coli NCCB 100282	Mueller Hinton agar (Oxoid)	37 °C, 48 h, aerobic
Yersinia ruckeri NCIM 13282	Plate-count agar (Difco), pH 6.5	30 °C, 24–26 h, aerobic
Gram positive		
Listeria monocytogenes ATCC 13929	Mueller Hinton agar (Oxoid)	37 °C, 48 h, aerobic
Bacillus cereus ATCC 11778	Iso-Sensitest Agar (Oxoid), pH 6 + 625 μg L^{-1} CAP	30 °C, 24–26 h, aerobic
Kocuria rhizophila	Iso-Sensitest Agar (Oxoid), pH 8	37 °C, 24–26 h, aerobic
Bacillus subtilis BGA	Plate-count agar (Difco), pH 8	37 °C, 24–26 h, aerobic
Bacillus pumilus CV 607	DST-agar (Oxoid), pH 7	37 °C, 24–26 h, aerobic

in total polyphenolic and total anthocyanin content may result in significantly different AB, AO and other properties of the plant products. Therefore, it is important to conduct the selection among cultivars and plant parts to choose those with the highest beneficial properties.[21]

The aim of this study was to gain comparable information about *in vitro* AB and radical scavenging activities of different plant species and their different parts. The more successive aim was to select plant materials for further use as antimicrobials or AO compounds in foods. Results of earlier studies were reviewed, and plant species and their varieties with multiple beneficial capacities and high horticultural relevance in northern Europe were selected. In particular, the highest anthocyanin content of the cultivated berries and high AB or AO activity of other plant parts were taken into account. Ethanol concentrations of 20% and 96%in the infusions were chosen to compare the summary effects of hydrophilic and more hydrophobic polyphenol complexes.

To our knowledge, this is the first study where ethanol infusions of the above-mentioned plants and their different parts were comparatively analyzed for AB and AO activities.

EXPERIMENTAL

Plant material

The planting material of rhubarb varieties was obtained from the collection of Pūre Horticultural Research Centre, Latvia. All studied plants were grown in the plantation of Polli Horticultural Research Centre, Estonia (58° 06′ N, 25° 32′ E). Two dark-rooted rhubarbs ('Victoria' and seedling 303) and one light-rooted ('Ogres') rhubarb were selected among 16 different cultivars or seedlings, according to the content of hydroxyanthraquinones. Berries of chokeberry (selected among three seedlings), blue honeysuckle (haskap berry) 'Tomitška' (selected among five cultivars) and blackcurrant 'Ben Alder' (selected among 37 cultivars); leaves of blackcurrant 'Pamyati Vavilova' and petioles of the above-mentioned garden rhubarbs were freeze dried using a VirTis AdVantage 2.0 EL freeze-dryer (SP Industries, Warminster, PA, USA) and kept at a temperature −40 °C until powdering. Roots of garden rhubarb cultivars and seedlings were washed, diced and dried at 50 °C in a drying oven (Binder FED101, Binder GmbH, Tuttlingen, Germany) and kept at room temperature.

Sample preparation and chemical analysis

All dried plant materials were powdered with a blender (Stollar/Kinetix® Control) to a particle size diameter of ≤3 mm; the necessary fraction was obtained with an analytical sieve shaker (AS300 control, Retsch GmbH, Germany). For the infusions, 1 g of each powder in duplicate was mixed with 20 mL of 20% and 96% aqueous ethanol. The mixtures were rotated on a Multi RS-60 Multirotator (Biosan, Riga, Latvia) at 40 rpm for 24 h at room temperature, followed by centrifugation at 2594 × *g* for 10 min on a Sigma 4-16KS (Sigma Laborzentrifugen GmbH, Germany) centrifuge. The supernatants were collected and further diluted by two, four and eight times for the estimation of AB and AO properties, and for quantitation of total polyphenol content (TPC)

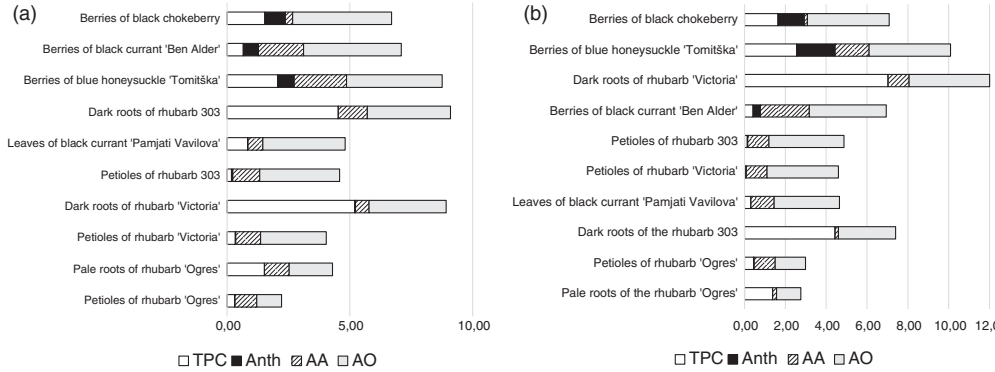


Figure 1. Chemical content and properties (g L^{-1}) of plant infusions (1:20, w/v): total polyphenols (TPC), total anthocyanins (Anth.), ascorbic acid (AA) and antioxidativity (AO) in rutin equivalents (g L^{-1}) of 20% ethanol infusions (a) and of 96% ethanol infusions (b). Bars are listed in descending order of AO.

and total anthocyanins. In addition, *trans*-rhapontin (Merck), rutin (Sigma), *trans*-resveratrol (Sigma) and emodin (Sigma) as single phenolic compounds were included in the AB and AO studies at four concentrations: 0.125, 0.25, 0.5 and 1 g L^{-1}. TPC and anthocyanin content of plant infusions were estimated by areas under high-performance liquid chromatography–UV chromatographic curves at 280 and 520 nm, respectively,[22] using an ultra-high-performance liquid chromatographic–mass spectrometric Shimadzu Nexera X2 system (Shimadzu Scientific Instruments, Kyoto, Japan). For the estimation of TPC and anthocyanin content, chlorogenic acid (Aldrich) and cyanidin 3-*O*-glucoside chloride (kuromanin chloride, Sigma) calibration curves were used, respectively. The qualitative analysis of plant extracts and the content of ascorbic acid (AA) and citric acid (CA) were performed with a 1100 Series LC/MSD Trap-XCT (Agilent Technologies, Santa Cruz, CA, USA) using AA (Sigma) and CA (Sigma) as calibration standards.[17] Total acidity and total sugar content were estimated with a Fourier transform infrared spectrometer (Bruker ALPHA ATR Platinum system; Bruker Optics GmbH, Germany). The AO of the infusions was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method,[23] and AO activities were expressed in rutin equivalents (g L^{-1}). Additionally, pH values of the 20% ethanol infusions were measured.

Bacterial strains

AB effect of plant infusions was determined against Gram-negative *Campylobacter jejuni* ATCC 33291, *Salmonella* Enteritidis ATCC 13076, *Escherichia coli* NCCB 100282, *Yersinia ruckeri* NCIM 13282

Table 2. Qualitative composition of the studied plants' 20% ethanol extracts, analyzed with negative and/or positive ion mode

Peak no.	Most abundant compounds in *Ribes nigrum* leaves	$[M-H]^-$ fragments
1	**Catechin gallate**	**305/179;219;261;137**
2	**Chlorogenic acid I**	**353/191;179**
3	Dihydroferulic acid rhamnoside	341/195;163;129
4	**Chlorogenic acid II**	**353/191**
5	Ferulic acid derivative	399/193;301
6	Coumarylquinic acid	337/191
7	Coumaroylquinic acid pentoside	675/337;191
8	**Myricetin glucoside**	**479/317;179;151**
9	**Quercetin-3-rutinoside syn. rutin**	**609/301**
10	**Quercetin glucoside**	**463/301**
11	**Quercetin acetylglucoside**	**505/301**
12	Kaempferol rutinoside	593/285
13	Kaempferol-3-*O*-glucoside	447/285
14	Kaempferol acetylglucoside	489/285
15	Isorhamnetin acetylglucoside	519/315
16	Chrysophanol glucoside	415/373;355
17	Oxylipin	327/311;211;171
18	Oxylipin 9*S*,12*S*,13*S*-trihydroxy-10*E*-octadecenoic acid (9,12,13-TriHOME)	329/311;211;171
Peak no.	Most abundant compounds in *Rheum rhaponticum* roots	$[M-H]^-$ fragments
1	**Procyanidin B1**	**577/407;289**
2	**Catechin**	**289/245**
3	**Epicatechin**	**289/245**
4	Piceatannol-*O*-glucoside 1	405/243
5	Resveratrol-*O*-glucoside	389/227
6	Piceatannol-*O*-glucoside 2	405/243
7	Piceid	389/227
8	Piceatannol	243/225
9	Rhapontigenin-*O*-glucoside 1	419/257
10	Rhapontigenin-*O*-glucoside 2	419/257
11	Rhapontigenin-*O*-glucoside 3	419/257
12[a]	Aloe-emodin-*O*-glucoside	431/269
13	Rhapontigenin	257/241
14[a]	Torachryson-*O*-glucoside	407/245
15[a]	Emodin-*O*-glucoside	431/269
16	Deoxyrhapontigenin-*O*-galloylglucosde	555/313;169
17[a]	Torachrysone-*O*-acetylglucoside	449/245
18[a]	Chrysophanol-*O*-glucoside	415/253
19[a]	Rhein-*O*-glucoside	445/283
20[a]	Chrysophanol-*O*-acetylglucoside	457/253
21	Deoxyrhapontigenin	241/226
22	Resveratrol dimer	453/453

Table 2. continued

Peak no.	Most abundant compounds in *Rheum rhaponticum* petioles	[M−H]$^-$ fragments	[M+ H]$^+$ fragments
1	Citric acid	191/111;173	
2	**Gallic acid**	**331/169**	
3	**Catechin**	**289/245**	
4	Paracoumaric acid glucoside	325/145	
5	Ferulic acid glucoside	355/193	
6	**Epicatechin**	**289/245**	
7	**Myricetin glucuronide**	**493/317;179**	
8	**Cyanidin-3-*O*-glucoside**		**449/287**
9	**Cyanidin-3-*O*-rutinoside**		**595/287**
10	**Myricetin rutinoside**	**625/317**	
11	Taxifolin glucoside	465/303;151	
12	**Epigallocatechin gallate or gallocatechin gallate**	**441/289**	
13	**Myricetin rhamnoside**	**463/317**	
14	**Rutin**	**609/301**	
15	**Quercetin glucuronide**	**477/301**	
16	**Quercetin rhamnoside**	**447/301**	
17	Kaempferol rutinoside	593/285	
18	Phloridzin	435/273	
19	**Myricetin glucoside glucuronide**	**479/316**	
20	Deoxyrhapontin	403/241	
21	**Quercetin glucoside**	**463/301**	
22	9*S*,12*S*,13*S*-trihydroxy-10*E*-octadecenoic acid (9,12,13-TriHOME)	329/171;229	

Peak no.	Most abundant compounds in *Ribes nigrum* berries	[M−H]$^-$ fragments	[M+H]$^+$ fragments
1	**Chlorogenic acid**	**353/191;179**	
2	Caffeic acid-*O*-glucoside	345	
3	Coumaryl quinic acid	(341)/179;161	
4	**Delphinidin-3-*O*-glucoside**	337/191	**465/303**
5	**Delphinidin-3-*O*-rutinoside**		**611/465;303**
6	**Cyanidin-3-*O*-glucoside**		**449/287**
7	**Cyanidin-3-*O*-rutinoside**		**595/287**
8	**Isorhamnetin-3-*O*-rutinoside**		**625/317**
9	**Myricetin-*O*-glucoside**		**481/319**
10	**Rutin**	**609/301**	**611/303**

Peak no.	Most abundant compounds in *Aronia melanocarpa* berries	[M−H]$^-$ fragments	[M+H]$^+$ fragments
1	**Chlorogenic acid I**	**353/191;179**	**355/163**
2	**Cyanidin3,5-di-*O*-glucoside**		**611/287**
3	**Chlorogenic acid II**	**353/191;179**	**355/163**
4	**Cyanidin-3-*O*-glucoside**		**449/287**
5	**Cyanidin-3-*O*-α-arabinopyranoside**		**419/287**
6	**Cyanidin-3-*O*-α-arabinopyranoside**		**419/287**
7	**Delphinidin-3-*O*-(2″-*O*-β-xylopyranosyl)-β-glycopyranoside**		**596/303**
8	Eriodictyol-7-*O*-β-glycuronide		465/289
9	**Rutin**	**609/301**	**611/303**
10	**Delphinidin-3-*O*-glucopyranoside**		**465/303**

Peak	Most abundant compounds in *Lonicera caerulea* berries	[M−H]$^-$ fragments	[M+H]$^+$ fragments
1	**Cyanidin3,5-di-*O*-glucoside**	0	**611/449;287**
2	**Cyanidin3,5-di-*O*-glucoside isomer**	**353/191;179**	**611/449;287**
3	**Chlorogenic acid**		**355/163**
4	**Cyanidin-3-*O*-glucoside**		**449/287**
5	**Cyanidin 3-*O*-rutinoside**		**595/287**
6	**Peonidin 3-*O*-glucoside**		**463/301**
7	**Quercetin *O*-rhamnoside-*O*-glucoside**		**609/463**

[a] Compound is better extractable with 96% ethanol compared to 20% ethanol.
The most potent antioxidative compounds (unpublished data) are marked in bold. Peak numbers refer to Fig. 2

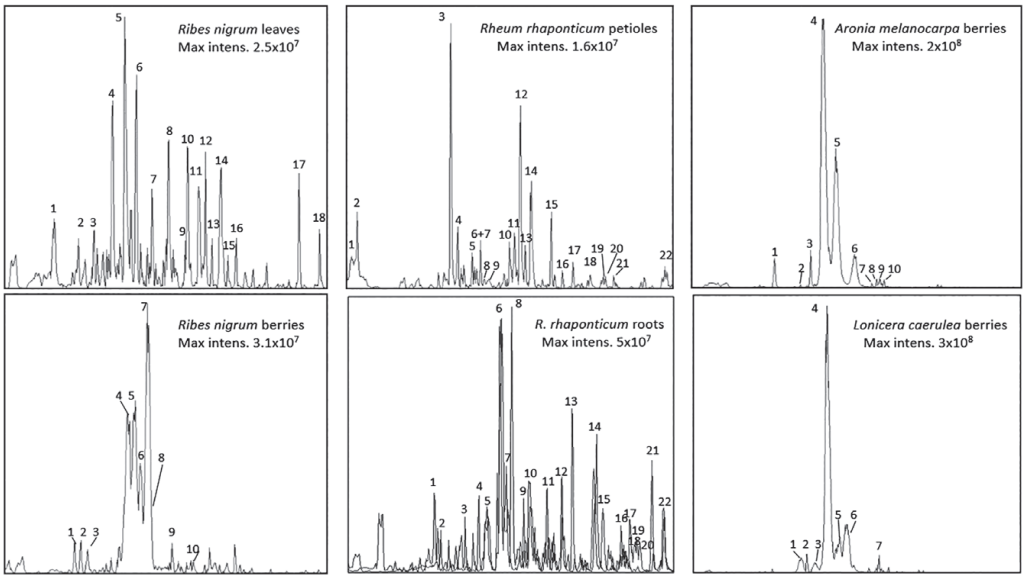


Figure 2. Base peak chromatograms of the studied plant extracts in 20% ethanol. Peak numbers are described in Table 2.

and Gram-positive *Listeria monocytogenes* ATCC 13929, *Bacillus cereus* ATCC 11778, *Kocuria rhizophila* ATCC 9341, *Bacillus subtilis* BGA and *Bacillus pumilus* CV 607 bacteria, obtained from the collections of the Estonian Veterinary and Food Laboratory and the Chair of Food Hygiene and Veterinary Public Health of Estonian University of Life Sciences.

Antibacterial activity (AB) test

AB activity testing was performed by modified agar well-diffusion method as previously described by Raudsepp *et al.*[17] In the case of *C. jejuni, L. monocytogenes, S.* Enteritidis and *E. coli*, suspensions with a final density of 10^5-10^6 mL^{-1} were prepared and, using sterile swabs, transferred uniformly on to the agar surface; for *C. jejuni* a sterile spatula was used. In the case of *B. cereus*, *Y. ruckeri*, *K. rhizophila*, *B. subtilis* and *B. pumilus*, a definite amount of incubated bacterial suspension was mixed with 400 mL sterilized and cooled (to 45 °C) Mueller-Hinton agar (Oxoid), to obtain a final density of 10^5-10^6 cfu mL^{-1} and then poured on to Petri dishes for the solidification at room temperature. Thereafter, the wells (5 mm in diameter) were made into agar gel using sterile tools. Subsequently, the wells were filled with 30 µL plant ethanol infusion in four different dilutions: 1:20 (w/v), 1:40, 1:80 and 1:160. Plates were incubated under conditions described in Table 1, the diameter of the inhibition zone (mm) was measured and the AB effect of a plant ethanol infusion was calculated as a mean of duplicate tests. As negative controls, 20% and 96% ethanol were used, and as a positive control chloramphenicol (LAB M; 1000 mg L^{-1}) was used.

Statistical analysis

MS Excel 2013 software was used to evaluate correlations between different chemical properties and AB activities of the infusions. Correlation was considered strong if $r \geq \pm 0.65$, moderate if $r \geq \pm 0.41$ to ± 0.64 or weak if r was in the interval 0 to ± 0.4.

RESULTS AND DISCUSSION

Chemical composition of plant infusions

The TPC of the plant infusions (1:20, w/v) varied from 0.18 to 5.21 g L^{-1} in 20% ethanol infusions and from 0.06 to 7.03 g L^{-1} in 96% ethanol infusions, rhubarb petioles being the lowest and rhubarb 'Victoria' roots the highest in TPC (Fig. 1. a and b). The anthocyanin content varied from 0 to 0.83 g L^{-1} and from 0 to 1.87 g L^{-1} in 20% and 96% ethanol infusions respectively, chokeberry having the highest anthocyanin content in 20% ethanol and blue honeysuckle in 96% ethanol. The ascorbic acid content varied from 0.31 to 2.14 g L^{-1} in 20% ethanol and from 0.16 to 2.4 g L^{-1} in 96% ethanol, chokeberry having the lowest and blue honeysuckle the highest in 20% ethanol, and blackcurrant berries the highest AA content in 96% ethanol. The pH of studied infusions varied from 3.15 (blackcurrant berries) to 3.8 (petioles of rhubarb 'Victoria'). The infusions of the berries and the rhubarb petioles contained anthocyanins, whereas rhubarb roots and blackcurrant leaves did not (Fig. 1. a and b). It was noted that the dark-rooted rhubarb cultivars had more anthocyanins in their petioles than the light-rooted cultivars (Fig. 1. a and b). The total acidity was highest in the petioles of rhubarb 'Ogres' (8.7 g L^{-1}) and the sugar:acid ratio was the highest in chokeberry berries (6.3), followed by blue honeysuckle berries (4.4). Blue honeysuckle had the highest content of total sugars (24.3 g L^{-1}), which exceeded blackcurrant and chokeberry berries approximately by 6 g L^{-1}. Qualitative analysis of the plant extracts revealed that the polyphenolic composition of the plants differed notably, containing polyphenols with different properties (Table 2 and Fig. 2); hence there were some differences in AO and AB properties.

Table 3. Antibacterial activity of plant infusions against Gram-positive bacteria (inhibition zones (mm) ± standard deviation)

		B. cereus		*B. pumilus*		*B. subtilis*		*K. rhizophila*		*L. monocytogenes*	
Plant infusion	Conc. (w/v)	A	B	A	B	A	B	A	B	A	B
Dark roots of rhubarb 303	1:20	16	15.5 ± 2.1	11[a]	13.5 ± 0.7	11	14	11	13.5 ± 0.7	9	11.5 ± 0.7
	1:40	15	12.5 ± 0.7	8[a]	11.5 ± 0.7	–	12 ± 3	10	11 ± 2	7	10
	1:80	11	11.5 ± 0.7	8[a]	11.5 ± 2.1	–	9 ± 1.4	–	9 ± 2	–	8.5 ± 0.7
	1:160	10	9	–	9	–	8	–	9[a]	–	8
Petioles of rhubarb 303	1:20	11.5 ± 0.71	12	–	9[a]	–	10	9	10	–	12
	1:40	10	9.5 ± 0.7	–	8[a]	–	10[a]	–	9[a]	–	10
	1:80	10	10	–	7[a]	–	–	–	–	–	8.5 ± 0.7
	1:160	8	8	–	–	–	–	–	–	–	6
Pale roots of rhubarb 'Ogres'	1:20	10	10	–	10	–	–	–	–	–	12
	1:40	8	9	–	8	–	–	–	–	–	10
	1:80	7	8	–	–	–	–	–	–	–	8
	1:160	0	–	–	–	–	–	–	–	–	–
Petioles of light rhubarb 'Ogres'	1:20	10	11	–	–	–	–	–	8	–	8
	1:40	8.5 ± 0.7	10	–	–	–	–	–	–	–	7
	1:80	7.5 ± 0.7	9	–	–	–	–	–	–	–	6
	1:160	–	7	–	–	–	–	–	–	–	0
Roots of rhubarb 'Victoria'	1:20	13	14	9	11	9	13[a]	10[a]	12[a]	–	12
	1:40	12	12	8	9	9[a]	11[a]	8[a]	9[a] ± 2	–	8
	1:80	8	11	–	7	–	–	–	–	–	7
	1:160	–	10	–	–	–	–	–	–	–	6
Petioles of rhubarb 'Victoria'	1:20	12	10	–	11[a]	–	–	–	–	–	11 ± 2
	1:40	9	9	–	11[a]	–	–	–	–	–	9
	1:80	7	7	–	–	–	–	–	–	–	8
	1:160	–	–	–	–	–	–	–	–	–	7
Berries of blackcurrant 'Ben Alder'	1:20	12	10[a]	–	10 ± 2	–	9	8 ± 2[a]	9[a]	–	11 ± 2
	1:40	10	9[a]	–	–	–	7	–	–	–	7
	1:80	7	–	–	–	–	–	–	–	–	–
	1:160	–	–	–	–	–	–	–	–	–	–
Leaves of blackcurrant 'Pamjati Vavilova'	1:20	14	10.5 ± 0.7	8[a]	–	–	8[a]	–	–	–	10
	1:40	13	9	7[a]	–	–	–	–	–	–	9
	1:80	10	8	6[a]	–	–	–	–	–	–	9
	1:160	8	7	–	–	–	–	–	–	–	8
Berries of black chokeberry	1:20	10	12	–	10[a]	–	–	–	12[a]	–	10.5 ± 0.7
	1:40	8	10	–	10[a]	–	–	–	11[a]	–	8
	1:80	7	9	–	–	–	–	–	–	–	7
	1:160	–	7	–	–	–	–	–	–	–	6
Berries of blue honeysuckle 'Tomitška'	1:20	10[a]	10	–	9[a]	–	–	–	–	–	–
	1:40	–	8	–	8[a]	–	–	–	–	–	–
	1:80	–	7	–	7[a]	–	–	–	–	–	–
	1:160	–	–	–	7[a]	–	–	–	–	–	–
Control (–)		–	–	–	–	–	–	–	–	–	–
Control (+)		29 ± 2		28.5 ± 3		33 ± 2		37.5 ± 0.7		26.5 ± 2	

[a] bacteriostatic effect was detected.
–, no visible growth detected; A, 20% ethanol–plant infusion; B, 96% ethanol–plant infusion.

Table 4. Antibacterial activity of plant infusions against Gram-negative bacteria (inhibition zones (mm) ± standard deviation)

Plant infusion	Conc. (w/v)	*C. jejuni* A	*C. jejuni* B	*S.* Enteritidis A	*S.* Enteritidis B	*E. coli* A	*E. coli* B	*Y. ruckeri* A	*Y. ruckeri* B
Dark roots of rhubarb 303	1:20	–	18 ± 3	10[a]	11.5 ± 0.7	10[a]	11 ± 1.4	8	9[a]
	1:40	–	13.5 ± 0.7	–	10	–	9	7	8[a]
	1:80	–	12	–	8	–	8	–	–
	1:160	–	10	–	7	–	7	–	–
Petioles of rhubarb 303	1:20	–	12	–	11 ± 2	–	11.5 ± 0.7	17	16 ± 2
	1:40	–	11	–	9.5 ± 0.7	–	9	14	12
	1:80	–	10	–	9	–	8	9	9
	1:160	–	8	–	8	–	7.5 ± 0.7	–	–
Pale roots of rhubarb 'Ogres'	1:20	–	16	–	12	–	12	–	12
	1:40	–	15	–	10	–	10	–	10
	1:80	–	14	–	–	–	–	–	8
	1:160	–	10	–	–	–	–	–	–
Petioles of rhubarb 'Ogres'	1:20	–	12	–	12[a]	–	10	10[a]	14
	1:40	–	11	–	9[a]	–	9	8[a]	9
	1:80	–	–	–	8[a]	–	7	–	–
	1:160	–	–	–	–	–	–	–	–
Roots of rhubarb 'Victoria'	1:20	–	18 ± 2	8	11.5 ± 0.7	8	11.5 ± 0.7	14	9
	1:40	–	15	6	10	–	10 ± 2	10	8[a]
	1:80	–	13	–	9	–	9	–	–
	1:160	–	10	–	8	–	8	–	–
Petioles of rhubarb 'Victoria'	1:20	–	8	–	9	–	9	13 ± 3	8
	1:40	–	7	–	8	–	8	–	–
	1:80	–	–	–	7	–	7	–	–
	1:160	–	–	–	7	–	7	–	–
Berries of blackcurrant 'Ben Alder'	1:20	–	15 ± 2	–	10	–	10	10	12 ± 2
	1:40	–	13 ± 2	–	9	–	9	8[a]	9
	1:80	–	12	–	–	–	–	–	–
	1:160	–	12	–	–	–	–	–	–
Leaves of blackcurrant 'Pamjati Vavilova'	1:20	–	13	–	9.5 ± 0.7	–	11	12[a]	10[a]
	1:40	–	11	–	9	–	10	8[a]	8[a]
	1:80	–	10	–	8	–	9	–	–
	1:160	–	9	–	8	–	8	–	–
Berries of black chokeberry	1:20	10	15	–	9 ± 2	–	11	–	8[a]
	1:40	8	12	–	7	–	11	–	–
	1:80	–	10	–	7	–	11	–	–
	1:160	–	8	–	6	–	8	–	–
Berries of blue honeysuckle 'Tomitška'	1:20	15	14 ± 2	–	9	–	8.5 ± 0.5	9[a]	10
	1:40	10	13	–	7	–	7	7[a]	7
	1:80	8	8	–	–	–	–	–	–
	1:160	–	–	–	–	–	–	–	–
Control (–)		–	–	–	–	–	–	–	–
Control (+)			40 ± 2		31.5 ± 2.1		26 ± 2		34 ± 4

[a] bacteriostatic effect detected.
–, no visible growth detected; A, 20% ethanol–plant infusion; B, 96% ethanol–plant infusion.

Antibacterial (AB) effect

In vitro AB activities, in the form of the diameters of growth inhibition zones of selected plant infusions, were determined against both Gram-positive (Table 3) and Gram-negative (Table 4) bacteria. The results indicated a remarkable *in vitro* AB effect of several plant infusions (Fig. 3). In the case of 20% ethanol–plant infusions, Gram-negative bacteria were less susceptible than Gram-positive bacteria. This is in agreement with Goňi *et al.*,[24] who reported Gram-negative bacteria being generally less susceptible to different antibacterial agents due to the outer lipopolysaccharide membrane, which restricts the diffusion of hydrophilic compounds into the bacterial cell. However, Taguri *et al.*[25] concluded that the result of Gram staining did not correlate with the AB effect, and susceptibility of bacteria growing in Mueller–Hinton medium depended mostly on the particular bacterial species. In the present study it was found that the strongest AB activity of tested plant 96% ethanol infusions was against *C. jejuni*, which is a Gram-negative micro-aerobic bacterium, and also against *B.*

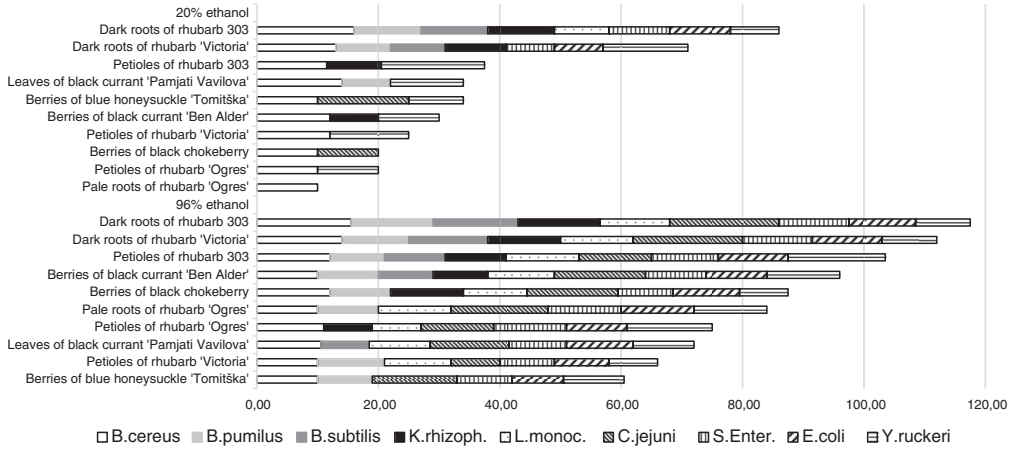


Figure 3. Bacterial growth inhibition zone diameters (mm) of plant infusions (1:20, w/v) in 20% and 96% ethanol, against each bacteria, listed in descending order of summarized AB activities.

cereus, which is a Gram-positive aerobic bacterium, with inhibition zones of 18 and 15.5 mm, respectively.

Against Gram-negative bacteria *C. jejuni*, *S.* Enteritidis and *E. coli*, the most effective at all tested dilutions were 96% ethanol infusions of the roots and petioles of the dark-rooted rhubarb 303 and the roots of 'Victoria' with inhibition zone diameters 7–18 mm (Fig. 3). Weaker AB effects of the same plant infusions against the above-mentioned bacteria were established in 20% ethanol (Fig. 3). Regarding *Y. ruckeri*, infusions of petioles of dark rhubarb 303 in 96% and 20% ethanol were equally effective (Table 4).

The most effective against all studied Gram-positive bacteria was 96% ethanol infusion of the roots of dark rhubarb seedling 303, and inhibition zone diameters were in the range of 8–15.5 mm at all dilutions. Among Gram-positive bacteria, *B. cereus* was the most susceptible to all ten tested plant infusions in 20% as well as in 96% ethanol (Fig. 2 and Table 3). It is notable that *L. monocytogenes*, which is known as a relatively resistant bacteria to different environmental factors, was susceptible to nine out of ten tested 96% ethanol infusions. Generally, more concentrated infusions (w/v) 1:20 or 1:40 had stronger AB or bacteriostatic effects against tested bacteria (Table 3).

It has been shown that solubility in water is a significant factor determining the extent to which hydrophobic compounds can be accumulated up to damaging lethal levels in bacterial cell phospholipid membranes.[24,26] In the current study, plant ethanol infusions were used; therefore, the mode of action of antibacterial agents cannot be explained solely by cell membrane damage.

trans-Resveratrol and emodin, both constituents of rhubarb roots, showed AB activity against Gram-negative *C. jejuni*, *S.* Enteritidis and *E. coli*, and against Gram-positive *L. monocytogenes* at their highest used concentration (1 g L^{-1}). Li *et al.*[27] have estimated by cell membrane permeability and flow cytometry assays the ability of hydrophobic emodin (the octanol–water partition coefficient log K_{ow} + 4.01; ECHA[28]) to destroy cell membrane integrity and increase membrane permeability; fluorescence spectroscopy assay had indicated the ability of emodin to influence conformation of membrane proteins in the case of Gram-positive *Haemophilus parasuis*. These mechanisms can possibly be used also to explain the AB effect against Gram-negative bacteria of rhubarb root 96% ethanol infusion that, in addition to emodin, contains several other relatively hydrophobic hydroxyanthraquinones.[9]

An important finding of the present study was that Gram-positive foodborne pathogenic bacteria *L. monocytogenes* and *B. cereus* as well as Gram-negative pathogens *C. jejuni*, *S.* Enteritidis and *E. coli* were inhibited by the ethanol infusions of the roots and petioles of rhubarb (both seedling 303 and 'Victoria'), which makes rhubarb a promising candidate for use as the source of natural antibacterials in food. In rhubarb, presumably hydroxyanthraquinones are the major active components, having many biological and pharmacological properties including AB activity.[29,30] In the study by Lu *et al.*,[2] the minimum inhibitory concentration (MIC) of crude extracts of rhubarb was positively related to hydroxyanthraquinone content, and similar to the results of the current study it was found that rhubarb may have potential use as an antibacterial agent for control of some pathogenic bacteria.

Free radical scavenging ability

The highest AO, expressed by DPPH free radical scavenging activity, was shown by chokeberry berries, with the highest content and variability of anthocyanins, both in 20% and 96% aqueous ethanol infusions, which is in agreement with the results of Tian *et al.*[21] Chokeberry was followed by 20% aqueous ethanol infusion of blackcurrant berries with the lowest total polyphenols and total anthocyanins among the berries. Obviously, AO properties of blackcurrant berries are primarily dependent on hydrophilic compounds such as ascorbic acid,[20] and subsequently on semi-polar anthocyanins[3,13] and flavon-3-ols – particularly rutin[31] – all of which are good antioxidants and more readily extractable with a more hydrophilic solvent (20% ethanol). Honeysuckle, with the highest total polyphenol content among berries and dark rhubarb roots (Fig. 1b) with the absolutely highest TPC, were also efficient. The AO properties of chokeberry, blackcurrant berries and blue honeysuckle berries were, however, very similar (Fig. 1. a). In the case of rhubarb roots, AO is obviously more dependent on relatively hydrophobic constituents such as the hydroxyanthraquinones emodin, aloe emodin and chrysophanol, as well as resveratrol dimers and trimers,[9,32] which are extractable

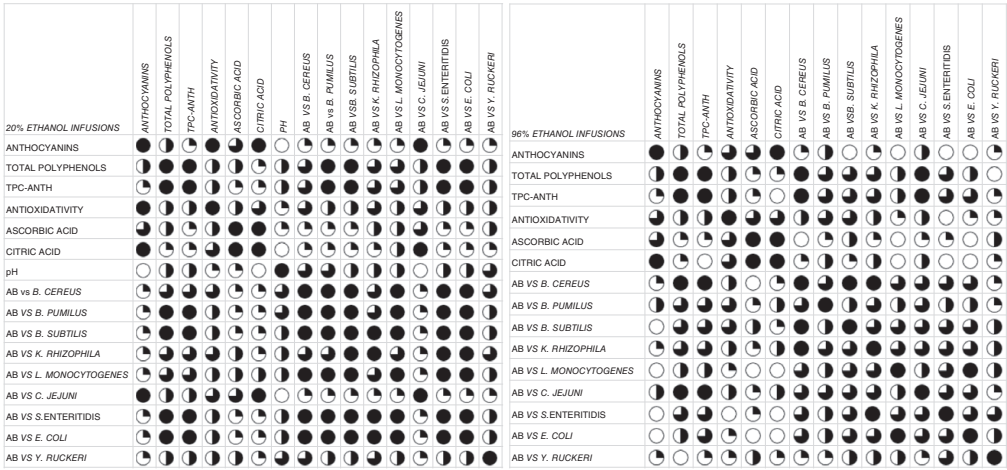


Figure 4. Correlations between characteristics of plant infusions in 20% and 96% ethanol solutions. TPC-Anth., total polyphenol content minus anthocyanin content; AB-antibacterial activity ●, strong positive correlation, $r \geq 0.65$; ○, strong negative correlation, $r \leq -0.65$; ◑, weak correlation, $r \sim 0$ to ± 0.4; ◕, moderate positive correlation, $r = 0.41$ to 0.64; ◔, moderate negative correlation, $r = -0.64$ to -0.41.

from the plant matrix with a more nonpolar solvent such as 96% ethanol. The content of anthocyanins was positively correlated with the AO of both 20% ($r = 0.65$) and 96% ($r = 0.47$) ethanol infusions of plants (Fig. 4), which is in the agreement with the results of Heinonen *et al.*[3] and Shih *et al.*[33] Also, content of citric and ascorbic acids, both outstanding transition metal chelators, had moderate positive correlation with the free radical scavenging activities of plant infusions (Fig. 4). It has been stated that organic acids, including citric acid, generally enhance the DPPH radical scavenging activity of ascorbic acid at a steady rate, whereas citric acid slows it down during the first minute of the reaction.[34] In the current study, the TPC in the plant infusions was weakly positively correlated with AO properties (Fig. 4), which may be caused by the high content of hydroxyanthraquinones and stilbenes in rhubarb root that have very strong AB[2,16] but weaker radical scavenging capacity.[32] In berries the bulk of the TPC were anthocyanins: strongly antioxidative molecules[3,33] but not equally good antibacterial compounds.

The AO properties of the studied polyphenol standard compounds were in descending order rutin > *trans*-resveratrol > *trans*-rhapontin > emodin, which is in agreement with Villaño *et al.*[35]

AB activity was more strongly correlated with total polyphenolic content of the plant infusions than with the total content of anthocyanins. On the other hand, the highest AO activity was determined for plant materials containing anthocyanins and ascorbic acid. The AB and AO of the studied plant infusions were not unambiguously correlated, indicating that different compounds may be involved in antioxidative properties compared to antibacterial properties.

A combination of powders of dark rhubarb roots and petioles with berries of black chokeberry, blackcurrant or some other anthocyanin-rich berries has outstanding potential as functional ingredients in food matrices.

CONCLUSIONS

The roots and petioles of rhubarb showed the highest AB activity against the studied bacteria, which highlights rhubarb as a promising candidate for use as a source of natural antibacterials in food, if possible contamination by soil microflora is reduced to minimum. The highest *in vitro* AB activities were measured for dark roots of rhubarb infusions in 96% ethanol. Also, *trans*-resveratrol and emodin, as single compounds – both present in rhubarb roots – revealed remarkable AB activity against the studied bacteria.

ACKNOWLEDGEMENTS

The research was financially supported by EU ERA-NET SUSFOOD project 'Sustainable plant ingredients for healthier meat products – proof of concepts' (SUSMEATPRO), and the basic financing project 'Metabolomics, antioxidative and antibacterial activity, and related mechanisms of food plants' of Estonian University of Life Sciences P170054VLTH.

The authors thank Liga Lepse from the Latvian University of Agriculture; and Hans Neppo, Liina Arus and Kersti Kahu from Polli Horticultural Research Centre, Estonian University of Life Sciences, for providing the plant material.

REFERENCES

1 Kovalenko K, Roasto M, Liepinš E, Mäesaar M and Hörman A, High occurrence of *Campylobacter* spp. in Latvian broiler chicken production. *Food Control* **29**:188–191 (2013).
2 Lu C, Wang H, Lv W, Xu P, Zhu J, Xie J *et al.*, Antibacterial properties of anthraquinones extracted from rhubarb against *Aeromonas hydrophila*. *Fish Sci* **77**:375–384 (2011).
3 Heinonen M, Antioxidant activity and antimicrobial effect of berry phenolics: a Finnish perspective. *Mol Nutr Food Res* **51**:684–691 (2007).
4 Skrovankova S, Sumczynski D, Mlcek J, Jurikova T and Sochor J, Bioactive compounds and antioxidant activity in different types of berries. *Int J Mol Sci* **16**:24673–24706 (2015).

5 He Z-H, He M-F, Ma S-C and But PP-H, Anti-angiogenic effects of rhubarb and its anthraquinone derivatives. *J Ethnopharmacol* **121**:313–317 (2009).
6 Neyrinck AM, Etxeberria U, Taminiau B, Daube G, Van Hul M, Everard A *et al.*, Rhubarb extract prevents hepatic inflammation induced by acute alcohol intake, an effect related to the modulation of the gut microbiota. *Mol Nutr Food Res* **61**:1–12 (2016).
7 Celli GB, Ghanem A and Brooks MS-L, A theoretical physiologically based pharmacokinetic approach for modeling the fate of anthocyanins *in vivo*. *Crit Rev Food Sci Nutr* **57**:3197–3207 (2017).
8 Stephan D, Schmitt A, Martins Carvalho S, Seddon B and Koch E, Evaluation of biocontrol preparations and plant extracts for the control of *Phytophthora infestans* on potato leaves. *Eur J Plant Pathol* **112**:235–246 (2005).
9 Püssa T, Raudsepp P, Kuzina K and Raal A, Polyphenolic composition of roots and petioles of *Rheum rhaponticum* L. *Phytochem Anal* **20**:98–103 (2009).
10 Raudsepp P, Kaldmäe H, Kikas A, Libek A-V and Püssa T, Nutritional quality of berries and bioactive compounds in the leaves of black currant (*Ribes nigrum* L.) cultivars evaluated in Estonia. *J Berry Res* **1**:53–59 (2010).
11 Vagiri M, Conner S, Stewart D, Andersson SC, Verrall S, Johansson E *et al.*, Phenolic compounds in blackcurrant (*Ribes nigrum* L.) leaves relative to leaf position and harvest date. *Food Chem* **172**:135–142 (2015).
12 Chaovanalikit A, Thompson MM and Wrolstad RE, Characterization and quantification of anthocyanins and polyphenolics in blue honeysuckle (*Lonicera caerulea* L.). *J Agric Food Chem* **52**:848–852 (2004).
13 Caprioli G, Iannarelli R, Innocenti M, Bellumori M, Fiorini D, Sagratini G *et al.*, Blue honeysuckle fruit (*Lonicera caerulea* L.) from eastern Russia: phenolic composition, nutritional value and biological activities of its polar extracts. *Food Funct* **7**:1892–1903 (2016).
14 Rop O, Mlcek J, Jurikova T, Valsikova M, Sochor J, Reznicek V *et al.*, Phenolic content, antioxidant capacity, radical oxygen species scavenging and lipid peroxidation inhibiting activities of extracts of five black chokeberry (*Aronia melanocarpa* (Michx.) Elliot) cultivars. *J Med Plants Res* **4**:2431–2437 (2010).
15 Tian Y, Liimatainen J, Alanne A-L, Lindstedt A, Liu P, Sinkkonen J *et al.*, Phenolic compounds extracted by acidic aqueous ethanol from berries and leaves of different berry plants. *Food Chem* **220**:266–281 (2017).
16 Kosikowska U, Smolarz HD and Malm A, Antimicrobial activity and total content of polyphenols of *Rheum* L. species growing in Poland. *Cent Eur J Biol* **5**:814–820 (2010).
17 Raudsepp P, Anton D, Roasto M, Meremäe K, Pedastsaar P, Mäesaar M *et al.*, The antioxidative and antimicrobial properties of the blue honeysuckle (*Lonicera caerulea* L.), Siberian rhubarb (*Rheum rhaponticum* L.) and some other plants, compared to ascorbic acid and sodium nitrite. *Food Control* **31**:129–135 (2013).
18 Hasper I, Ventskovskiy BM, Rettenberger R, Heger PW, Riley DS and Kaszkin-Bettag M, Long-term efficacy and safety of the special extract ERr 731 of *Rheum rhaponticum* in perimenopausal women with menopausal symptoms. *Menopause* **16**:117–131 (2009).
19 Zheng J, Yang B, Ruusunen V, Laaksonen O, Tahvonen R, Hellsten J *et al.*, Compositional differences of phenolic compounds between black currant (*Ribes nigrum* L.) cultivars and their response to latitude and weather conditions. *J Agric Food Chem* **60**:6581–6593 (2012).
20 Vagiri M, Ekholm A, Öberg E, Johansson E, Andersson SC and Rumpunen K, Phenols and ascorbic acid in black currant (*Ribes nigrum* L.): variation due to genotype, location and year. *J Agric Food Chem* **61**:9298–9306 (2013).
21 Tian Y, Puganen A, Alakomi H-L, Uusitupa A, Saarela M and Yang B, Antioxidative and antibacterial activities of aqueous ethanol extracts of berries, leaves, and branches of berry plants. *Food Res Int* **106**:291–303 (2018).
22 Kapp K, Hakala E, Orav A, Pohjala L, Vuorela P, Püssa T *et al.*, Commercial peppermint (*Mentha* × *piperita* L.) teas: antichlamydial effect and polyphenolic composition. *Food Res Int* **53**:758–766 (2013).
23 Helmja K, Vaher M, Püssa T, Raudsepp P and Kaljurand M, Evaluation of antioxidative capability of the tomato (*Solanum lycopersicum*) skin constituents by capillary electrophoresis and high-performance liquid chromatography. *Electrophoresis* **29**:3980–3988 (2008).
24 Goñi P, Lopez P, Sanchez C, Gomez-Lus R, Becerril R and Nerin C, Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. *Food Chem* **116**:982–989 (2009).
25 Taguri T, Tanaka T and Kouno I, Antibacterial spectrum of plant polyphenols and extracts depending upon hydroxyphenyl structure. *Biol Pharm Bull* **29**:2226–2235 (2006).
26 Cox SD, Mann CM and Markham JL, Interactions between components of the essential oil of *Melaleuca alternifolia*. *J Appl Microbiol* **91**:492–497 (2001).
27 Li L, Song X, Yin Z, Jia R, Li Z, Zhou X *et al.*, The antibacterial activity and action mechanism of emodin from *Polygonum cuspidatum* against *Haemophilus parasuis in vitro*. *Microbiol Res* **186–187**:139–145 (2016).
28 ECHA, European Chemicals Agency, *Registration Dossier. Anthraquinone. Toxicity to Soil Macroorganisms Except Arthropods* (2011). [Online]. Available: https://echa.europa.eu/registration-dossier/-/registered-dossier/5769/6/4/2/ [24 October 2017].
29 Iizuka A, Iijima OT, Kondo K, Itakura H, Yoshie F, Miyamoto H *et al.*, Evaluation of rhubarb using antioxidative activity as an index of pharmacological usefulness. *J Ethnopharmacol* **91**:89–94 (2004).
30 Wang J, Zhao H, Kong W, Jin C, Zhao Y, Qu Y *et al.*, Micro calorimetric assay on the antimicrobial property of five hydroxyanthraquinone derivatives in rhubarb (*Rheum palmatum* L.) to *Bifidobacterium adolescentis*. *Phytomedicine* **17**:684–689 (2010).
31 Afanas'ev IB, Dcrozhko AI, Brodskii AV, Kostyuk VA and Potapovitch AI, Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochem Pharmacol* **38**:1763–1769 (1989).
32 Yen G-C, Duh P-D and Chuang D-Y, Antioxidant activity of anthraquinones and anthrone. *Food Chem* **70**:437–441 (2000).
33 Shih P-H, Yeh C-T and Yen GC, Anthocyanins induce the activation of phase II enzymes through the antioxidant response element pathway against oxidative stress-induced apoptosis. *J Agric Food Chem* **55**:9427–9435 (2007).
34 Lo Scalzo R, Organic acids influence on DPPH scavenging by ascorbic acid. *Food Chem* **107**:40–43 (2008).
35 Villaño D, Fernández-Pachón MS, Moyá ML, Troncoso AM and García-Parrilla MC, Radical scavenging ability of polyphenolic compounds towards DPPH free radical. *Talanta* **71**:230–235 (2007).

CURRICULUM VITAE

First name Piret
Surname Raudsepp

ORCID 0000-0002-9447-1895
E-mail piret.raudsepp@emu.ee

Date of birth 04.05.1979

Institution Estonian University of Life Sciences, Institute of Veterinary Medicine and Animal Sciences, Chair of Food Hygiene and Veterinary Public Health, Kreutzwaldi 56/3, Tartu, Estonia

Position Junior researcher of food polyphenols chromatography, lecturer of human nutrition

Teaching Human nutritition, Food related myths and reality

Education

2007–2015	PhD student in Estonian University of Life Sciences, Institute of Veterinary Medicine and Animal Sciences, Chair of Food Hygiene and Veterinary Public Health
2005–2007	MSc Degree in Horticulture, Estonian University of Life Sciences, 2007
2000–2005	BSc in Biology, University of Tartu, 2005, now equal to European MSc degree

Career history

2017–...	Estonian University of Life Sciences, Chair of Food Hygiene and Public Health, junior researcher of food polyphenols chromatography
2010–...	Estonian University of Life Sciences, lecturer of human nutrition
2013–2017	Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Polli Horticultural Research Centre (PlantValor), researcher
2009–2013	Bio-Competence Centre of Healthy Dairy Products (BioCC), researcher

2005–2010	Estonian University of Life Sciences, Institute of Veterinary Medicine and Animal Sciences, HPLC specialist
2004–2005	LLC Latimeeria, art gallery assistant
2001	University of Tartu, field researcher (ecology)

Scientific-organisational and administrative activities

2017−...	Reviewer for the Journal of Integrative Medicine
2017−...	Reviewer for the Journal of the Science of Food and Agriculture
2016−...	Reviewer for the Journal of Separation Science
2016−...	Reviewer for the journal Current Nutrition & Food Science
2015−...	Reviewer for the journal Critical Reviews in Food Science and Nutrition
2015−...	Reviewer for the journal Agronomy Research
2013−...	Reviewer for the journal Industrial Crops and Products
2012−...	Reviewer for the journal International Journal of Food Engineering
2012−...	Member of Estonian Nutrition Recommendation working group

Field of research

1. Biosciences and Environment; 1.7. Food Sciences

Participation in research projects

2019−2021	"The antimicrobial effect of natural bioactive substances in food (P180279VLTR)", Mati Roasto, Estonian University of Life Sciences, Institute of Veterinary Medicine and Animal Sciences, Chair of Food Hygiene and Veterinary Public Health
2015−2019	"PlantValor – enhancing the scientific research and cooperation on valorization of plant origin raw materials, and increasing sectorial innovation capacity (F15130PKPA)", Piia Pääso, Aret Vooremäe, Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Polli Horticultural Research Centre
2017−2018	"Food plant metabolomics – intensity and mechanisms of antioxidant and antibacterial effect of secondary metabolites" (P170054VLTH), Mati Roasto, Estonian

	University of Life Sciences, Institute of Veterinary Medicine and Animal Sciences
2015−2018	"Sustainable plant ingredients for healthier meat products – proof of concepts" (8-2/T15024VLTH), Tõnu Püssa, Estonian University of Life Sciences, Institute of Veterinary Medicine and Animal Sciences, Institute of Agricultural and Environmental Sciences
2015−2017	Organizing the conference "Healthy Animal and Healthy Food 2016", publishing the conference proceedings (8-2/T15144VLVV), Riho Gross, Estonian University of Life Sciences, Institute of Veterinary Medicine and Animal Sciences
2013−2014	"Development of Epilobium angustifolium dry extract and flavoured oils" (8-2/T13188PKPA), Uko Bleive, Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Polli Horticultural Research Centre
2008−2011	"Diversity of bioactive compounds in fruits and berries" (ETF7703), Ave Kikas, Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences
2006−2011	"Improvement of assortment of fruit crops, maintenance of genetic diversity and development of environment-friendly cultivating methods II" (SF1092711s06), Asta-Virve Libek, Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences
2005−2008	"Influence of different cultivation methods on the productivity, crop quality and cultivation environment. Breeding of the blueberry and lingonberry varieties" (ETF6046), Taimi Paal, Estonian University of Life Sciences, Institute of Forestry and Rural Engineering

Honours & awards

2019	Eesti Maaülikooli „Ülikoolisisese koostöö auhind“ Projektimeeskond: Tõnu Püssa, Mati Roasto, Dea Anton, Piret Raudsepp, Uko Bleive, Hedi Kaldmäe
2014	COST delegate stipend to participate in conference Food structure and Design, Porto, Portugal
2014	NORDPLUS stipend to participate in course Prevention of Obesity, University of Helsinki

2014	The reward of the science in practice for the project "The product development experiment of the smoothie" Project team: Hedi Kaldmäe, Uko Bleive, Ann Ojarand, Pille Põllumäe, Piia Pääso, Piret Raudsepp
2013	COST delegate stipend to participate in PhD student course (milk bio peptides) in University College Dublin, Ireland
2011	Travel Grant of Kristjan Jaak to practice in University of Helsinki, Department of Food and Environmental Sciences
2011	The Stipend of the the City Council of Estonian University of Life Sciences
2010	ESF DoRa stipend, to attend an international course "Functional Foods" for doctoral students in University of Helsinki
2006	Travel grant of Kristjan Jaak to participate (poster presentation) in conference ICoMST, Dublin, Ireland

Self-improvement courses

Genes – myths and reality (P2TP.TK.072), University of Tartu, 2 EAP, 25.01−22.03.2021

Developing the health promoting foods, based on gut microbiome (AU.690), Estonian University of Life Sciences, (4h), 26.02.2021

E-learning experience seminar (P2OO.TK.087), University of Tartu, 0.25 EAP, 18.10.2018

Practical aspects of laboratory work, University of Tartu, 0.5 EAP, 20.−21.12.2017

Nitrate and nitrite in production and processing of agricultural products, Estonian Universty of Life Sciences, 10.11.2017

Food packaging, assurance of food safety and providing food related information to customers, Estonian University of Life Sciences, 22.09.2017

Needs and expectations of the consumer – key of the development of a successful product, Estonian University of Life Sciences 25.11.−07.12.2016

Nutrition and food supplement recommendations for the athlete, University of Tartu, 0.25 EAP, 26.08.2016

HACCP for food processing enterprise, Estonian University of Life Sciences, 23.01.2015

NORDPLUS course Prevention of Obesity – Community Approach, University of Helsinki, 18.−22.8.2014

Visualization of data and analyze results. Estonian University of Life Sciences, 27.−31.01.2014

Multifactorial statistics. Estonian Universty of Life Sciences, 12.−16.08.2013

Data processing and analyze with MS Excel. Estonian University of Life Sciences, 27.−31.05.2013

Teaching speciality subjects in English. Estonian University of Life Sciences, 10.09.2012−31.05.2013

Functional Foods, University of Helsinki, 30.05.–04.06.2010

ELULOOKIRJELDUS

Eesnimi Piret
Perenimi Raudsepp

ORCID 0000-0002-9447-1895
E-mail piret.raudsepp@emu.ee

Sünniaeg 04.05.1979

Töökoht Eesti Maaülikool, veterinaarmeditsiini ja loomakasvatuse instituut, toiduhügieeni ja rahvatervise õppetool, Kreutzwaldi 56/3, 51006, Tartu, Eesti

Ametikohad Toidu polüfenoolide kromatograafia nooremteadur, inimese toitumisõpetuse lektor

Õppetöö Inimese toitumisõpetus, toiduga seonduvad müüdid ja tegelikkus

Haridus

2007–2015 Eesti Maaülikool, veterinaarmeditsiini ja loomakasvatuse instituut, toiduhügieeni ja rahvatervise õppetool, doktoriõpe

2005–2007 Eesti Maaülikool, põllumajandus- ja keskkonnainstituut, teadusmagistriõpe

2000–2005 Tartu Ülikool, botaanika ja ökoloogia instituut/farmaatsia instituut, bakalaureuseõpe, nüüdseks võrdsustatud magistriõppega

Teenistuskäik

2017–... Eesti Maaülikool, toidu polüfenoolide kromatograafia nooremteadur

2010–... Eesti Maaülikool, inimese toitumisõpetuse lektor

2013–2017 Eesti Maaülikool, põllumajandus- ja keskkonnainstituut, Polli aiandusuuringute keskus (PlantValor), teadur

2010–2018 Eesti Maaülikool, veterinaarmeditsiini ja loomakasvatuse instituut, toiduhügieeni osakond, inimese toitumis-õpetuse lektor

2009–2013 OÜ Tervisliku Piima Biotehnoloogiate Arenduskeskus, teadur
2005–2010 Eesti Maaülikool, veterinaarmeditsiini ja loomakasvatuse instituut, kromatograafia spetsialist
2004–2005 Osaühing Latimeeria, galerist
2001 Tartu Ülikool, uurija välitöö rühmas (ökoloogia)

Teadusorganisatsiooniline ja -administratiivne tegevus

2017−... Ajakirja Journal of Integrative Medicine retsensent
2017−... Ajakirja Journal of the Science of Food and Agriculture retsensent
2016−... Ajakirja Journal of Separation Science retsensent
2016−... Ajakirja Current Nutrition & Food Science retsensent
2015−... Ajakirja Critical Reviews in Food Science and Nutrition retsensent
2015−... Ajakirja Agronomy Research retsensent
2013−... Ajakirja Industrial Crops and Products retsensent
2012−... Ajakirja International Journal of Food Engineering retsensent
2012−... Eesti toitumissoovituste uuendamine, töörühma liige

Teadustöö põhisuunad

1. Bio- ja keskkonnateadused; 1.7. Toiduteadused

Osalemine teadusprojektides

2019−2021 "Looduslike bioaktiivsete ainete toime ning seonduvate mehhanismide uurimine toidumaatriksites (P180279VLTR)", Mati Roasto, Eesti Maaülikool, Veterinaarmeditsiini ja loomakasvatuse instituut, Toiduhügieeni ja rahvatervise õppetool
2015−2019 "PlantValor taimse tooraine väärindamise alase teaduskoostöö ja innovatsioonivõimekuse tõstmine (F15130PKPA)", Piia Pääso, Aret Vooremäe, Eesti Maaülikool, põllumajandus- ja keskkonnainstituut, Polli aiandusuuringute keskus
2017−2018 „Toidutaimede metaboloomika ning sekundaarsete metaboliitide antioksüdantse ja antibakteriaalse toime intensiivsuse ja mehhanismide uurimine“ (P170054VLTH), Mati Roasto, Eesti Maaülikool, veterinaarmeditsiini ja loomakasvatuse instituut

2015−2018 „Säästvad taimsed lisandid tervislikumate lihatoodete saamiseks – ideede tõestamine“ (8-2/T15024VLTH), Tõnu Püssa, Eesti Maaülikool, veterinaarmeditsiini ja loomakasvatuse instituut, põllumajandus- ja keskkonnainstituut

2015−2017 „Konverentsi „Terve loom ja tervislik toit 2016“ korraldamine ja konverentsikogumiku väljaandmine (8-2/T15144VLVV), Riho Gross, Eesti Maaülikool, veterinaarmeditsiini ja loomakasvatuse instituut

2013−2014 „Kuivekstraktide ja maitseõlide tootearendusprojekt“ (8-2/T13188PKPA), Uko Bleive, Eesti Maaülikool, põllumajandus- ja keskkonnainstituut, Polli aiandusuuringute keskus

2008−2011 „Bioaktiivsete ühendite mitmekesisus puuviljades ja marjades“ (ETF7703), Ave Kikas, Eesti Maaülikool, põllumajandus- ja keskkonnainstituut

2006−2011 „Aiakultuuride sortimendi parandamine, geneetilise mitmekesisuse säilitamine ja keskkonnasäästliku viljelustehnoloogia arendamine II“ (SF1092711s06), Asta-Virve Libek, Eesti Maaülikool, põllumajandus- ja keskkonnainstituut

2005−2008 „Erinevate kultiveerimisviiside mõju metsamarjade produktiivsusele, saagi kvaliteedile ja keskkonnale. Mustika- ja pohlasortide aretus“ (ETF6046), Taimi Paal, Eesti Maaülikool, metsandus- ja maaehitusinstituut

Loometöö

„Milline mahl on tervislik“, Kodutohter 2015, nr 2

„Taimeekstraktid leiavad tee nii kreemipurki kui toidulauale“, Postimees, Maaelu Edendaja, 5/9, 12.05.2014

Õpiobjekt „Toiduvalgud“, https://toiduvalgud.wordpress.com/

Õpiobjekt „Funktsionaaltoidud“, https://funktsionaaltoidud.wordpress.com/

Valged mustikad ja valge põdrakanep, Eesti Loodus 2/2006

Teaduspreemiad ja tunnustused

2019 Eesti Maaülikooli „Ülikoolisisese koostöö auhind“ Projektimeeskond: Tõnu Püssa, Mati Roasto, Dea Anton, Piret Raudsepp, Uko Bleive, Hedi Kaldmäe

2014 COST delegaadi stipendium osalemiseks konverentsil Food Structure and Design, Porto, Portugal

2014	NORDPLUSi stipendium osalemiseks kursusel Prevention of Obesity. Helsingi Ülikool
2014	Eesti Maaülikooli rakendusteaduste preemia, projekt „Tootearenduskatse smuuti väljatöötamiseks". Projektimeeskond: Hedi Kaldmäe, Uko Bleive, Ann Ojarand, Pille Põllumäe, Piia Pääso, Piret Raudsepp
2013	COST delegaadi stipendium osalemiseks piimabiopeptiidide teemalisel doktorantide kursusel Dublini Ülikooli Kolledžis Iirimaal
2011	Kristjan Jaagu välissõidu stipendium Helsingi Ülikooli toidu ja keskkonnateaduste laboratooriumis praktiseerimiseks
2011	Eesti Maaülikooli Raefondi stipendium
2010	ESF DoRa stipendium, rahvusvahelisel doktorantide kursusel „Functional Foods" osalemiseks Helsingi Ülikoolis Soomes
2006	Kristjan Jaagu välissõidu stipendium osalemiseks konverentsil ICoMST Dublinis, Iirimaal, plakatekandega

Enesetäiendus

Geenid - müüdid ja tegelikkus (P2TP.TK.072), Tartu Ülikool, 2 EAP, 25.01-22.03.2021

Tervist toetava toidu arendus seedetrakti mikrobioomi põhjal (AU.690), Eesti Maaülikool, (4h), 26.02.2021

E-õppega edasi. E-õppe kogemusseminar P2OO.TK.087, Tartu Ülikool, 0,25 EAP, 18.10.2018

Laboritöö praktilised aspektid, Tartu Ülikool, 0,5 EAP, 20.−21.12.2017

Nitraat ja nitrit põllumajandustoodete tootmisel ja töötlemisel, EMÜ, 10.11.2017

Toidu pakendamine, toiduohutuse tagamine ja toidualase teabe esitamine tarbijale, EMÜ, 22.09.2017

Tarbijate vajadused ja ootused – võti eduka toote arendamiseks, EMÜ, 25.11.–07.12.2016

Toitumis- ja toidulisandite soovitused sportijale (P2AV.TK.784), Tartu Ülikool, 26.08.2016

Toidukäitlemisettevõtte enesekontrollisüsteem, EMÜ, 23.01.2015

NORDPLUS kursus Prevention of Obesity – Community Approach, Helsingi Ülikool, 18.−22.8.2014

Andmete ja analüüsitulemuste esitamine ja visualiseerimine, EMÜ, 27.−31.01.2014

Mitmemõõtmeline statistika, EMÜ, 12.−16.08.2013

Andmete haldus ja analüüs MS Excelis, EMÜ, 27.−31.05.2013
Eriala õpetamine inglise keeles, EMÜ, 10.09.2012−31.05.2013
Kursus Functional Foods, Helsingi Ülikool, 30.05.2010−04.06.2010

LIST OF PUBLICATIONS

1.1. Scholarly papers indexed by Web of Science Science Citation Index Expanded, Social Sciences Citation Index, Arts & Humanities Citation Index and/or indexed by Scopus

Laurson, P., **Raudsepp, P.**, Kaldmäe, H., Kikas, A. and Mäeorg, U., 2020. The deconvolution of FTIR-ATR spectra to five Gaussians for detection of small changes in plant–water clusters. AIP Advances, 10, 085214.

Raudsepp, P., Koskar, J., Anton, D., Meremäe, K., Kapp, K., Laurson, P., Bleive, U., Kaldmäe, H., Roasto, M., Püssa, T., 2019. Antibacterial and antioxidative properties of different parts of garden rhubarb, blackcurrant, chokeberry and blue honeysuckle. Journal of the Science of Food and Agriculture, 99, 2311−2320.

Anton, D., Koskar, J., **Raudsepp, P**., Meremäe, K., Kaart, T., Püssa, T., Roasto, M., 2019. Antimicrobial and antioxidative effects of plant powders in raw and cooked minced pork. Foods, 8, 661.

Heinmaa, L., Moor, U., Põldma, P., **Raudsepp, P.,** Kidmaose, U., Lo Scalzo, R., 2017. Content of health-beneficial compounds and sensory properties of organic apple juice as affected by processing technology. LWT- Food Science and Technology, 85, 372−379.

Anton, D., **Raudsepp, P.,** Roasto, M., Meremäe, K., Kuusik, S., Toomik, P., Elias, P., Laikoja, K., Kaart, T., Lepiku, M., Püssa, T., 2016. Comparative study of microbiological, chemical and sensory properties of kefirs produced in Estonia, Latvia and Lithuania. Journal of Dairy Research, 83, 89−95.

Raudsepp, P., Anton, D., Roasto, M., Meremäe, K., Pedastsaar, P., Mäesaar, M., Raal, A., Laikoja, K., Püssa, T., 2013. The antioxidative and antimicrobial properties of the blue honeysuckle (*Lonicera caerulea* L.), Siberian rhubarb (*Rheum rhaponticum* L.) and some other plants, compared to ascorbic acid and sodium nitrite. Food Control, 31, 129−135.

Raudsepp, P., Kaldmäe, H., Kikas, A., Libek, A.-V., Püssa, T., 2010. Nutritional quality of berries and bioactive compounds in the leaves of black currant (*Ribes nigrum* L.) Cultivars evaluated in Estonia. Journal of Berry Research, 1, 53−59.

Püssa, T., **Raudsepp, P**., Toomik, P., Pällin, R., Mäeorg, U., Kuusik, S., Soidla, R., Rei, M., 2009. A study of oxidation products of free

polyunsaturated fatty acids in mechanically deboned meat. Journal of Food Composition and Analysis, 22, 307−314.

Püssa, T., **Raudsepp, P**., Kuzina, K., Raal, A., 2009. Polyphenolic composition of roots and petioles of *Rheum rhaponticum* L. Phytochemical Analysis, 20, 98−103.

Püssa, T., Pällin, R., **Raudsepp, P.,** Soidla, R., Rei, M., 2008. Inhibition of lipid oxidation and dynamics of polyphenol content in mechanically deboned meat supplemented with sea buckthorn (*Hippophae rhamnoides*) berry residues. Food Chemistry, 107, 714−721.

Helmja, K., Vaher, M., Püssa, T., **Raudsepp, P.**, Kaljurand, M., 2008. Evaluation of antioxidative capability of the tomato (*Solanum lycopersicum*) skin constituents by capillary electrophoresis and high-perfoemance liquid chromatography. Electrophoresis, 29, 3980−3988.

1.2. Peer-reviewed papers in other international research journals with an ISSN code and international editorial board, which are circulated internationally and open to international contributions

Roasto, M., Meremäe, K., **Raudsepp, P.**, Anton, D., Pedastsaar, P., Elias, T., Mäesaar, M., Matt, D., 2012. The inhibitory effect of plant infusions on selected bacteria. Chemine technologija, 61, 14−18.

2.2. Monographs

Kikas, A., Libek, A.-V., Kelt, K., **Raudsepp, P**., Kahu, K., Vahenõmm, K., Pennar, M., 2008. Black currant cultivation. Estonian University of Life Sciences, Tartu, Eesti Loodusfoto.

2.5. Published reports of a scientific project or a scientific analyse

Pitsi, T.; Zilmer, M.; Vaask, S.; Nurk, E.; Kuu, S.; Maser, M.; Lõhmus, K.; Ehala-Aleksejev, K.; Raukas, R.; Sooba, E; Villa, I; Parts, L.; Lindsaar, M.; Sammel, A.; Radin, M.; Einberg, O.; Simm-Pärle, P.; Lamp, H.; Hiis, T.; Rajasalu, P; **Raudsepp, P**…Potisepp, S., 2017. Nutrition and exercise recommendations in Estonia 2015. Tallinn.

3.2. Published conference presentations

Püssa, T., Pällin, R., **Raudsepp, P**., Toomik, P., Veri, K., Soidla, R., Rei, M., 2007. Use of bilberry sauce for inhibition of fatty acid oxidation during marination of pork. Proceedings of 53rd International Congress of Meat Science and Technology, 443−444, China Agricultural University Press, Beijing, China 05.−10.08. 2007.

3.4. Papers/presentations, published in conference proceedings

Raudsepp, P., Anton, D., Meremäe, K., Kapp, K., Roasto, M., Püssa, T., 2018. Antibacterial and antioxidative properties of different plant parts. Euroindoamerican Natural Products Meeting, Madrid, Spain. Book of abstracts: EIAMNP, Euroindoamerican Natural Products Meeting (Congress), 133−133, Spanish Research Council Madrid, Spain, 29.05−1.06. 2018.

Anton, D., Koskar, J., **Raudsepp, P.**, Meremäe, K., Püssa, T., Roasto, M., 2018. Antimicrobial and antioxidative effects of plant powders in minced pork. EIAMNP, Euroindoamerican Natural Products Meeting, Madrid, Spain. Book of abstracts, 134−134, Spanish Research Council Euroindoamerican Natural Products Meeting (Congress), Madrid, Spain, 29.05−1.06.2018.

Raudsepp, P., Anton, D., Meremäe, K., Püssa, T., Roasto, M., 2018. Extracts of different plant parts; their antibacterial and antioxidative properties. Abstract book referenced on the Archives of International Society of Antioxidants in Health & Nutrition, Volume 6- Issue 3, 142−142. Bonn Polyphenols World Congress, University of Bonn, Germany, September 25.−28.09. 2018.

Anton, D., **Raudsepp, P**., Meremäe, K., Püssa, T., Roasto, M., 2018. Antimicrobial and antioxidative effects of rhubarb, tomato and berry powders in raw and cooked pork. Abstract book referenced on the Archives of International Society of Antioxidants in Health & Nutrition, Volume 6- Issue 3, 77−77. Bonn Polyphenols World Congress, University of Bonn, Germany, 25.−28.09. 2018.

Raudsepp, P., Anton, D., Pedastsaar, P., Roasto, M., Meremäe, K., Püssa, T., 2011. The evaluation of antioxidant effect of six plant infusions compared to the ascorbic acid. Programme and materials: 5th International Conference on Polyphenols and Health (5th ICPH), 182−182, University of Barcelona, Sitges, Barcelona, Spain, 17.−20.10. 2011.

3.5. Papers/presentations, published in local conference proceedings

Raudsepp, P., Anton, D., Roasto, M., Meremäe, K., Püssa, T., 2019. Overview of the results of the ERA-NET SUSFOOD international project SUSMEATPRO: Vegetable Additives to Meat Products. Healthy Animal and Healthy Food, Estonian University of Life Sciences, Tartu, 70−72, Vali Press OÜ.

Raudsepp, P., Anton, D., Roasto, M., Meremäe, K., Püssa, T., 2018. The comparison of the antibacterial and antioxidative properties of different plants. Kass, Marko (Toim.). Conference proceedings: Healthy Animal and Healthy Food, Estonian University of Life Sciences, Tartu, 35−40. Tartu, Vali Press.

Raudsepp, P., Libek, E., Rätsep, R., Kikas, A., Libek, A.-V., Arus, L., Kaldmäe, H., Bleive, U., 2017. Antotsüaanide sisalduse varieeruvus ja säilimine mõnedes Eestimaal kasvavates marjades. Conference proceedings: Healthy Animal and Healthy Food, Estonian University of Life Sciences, Tartu, 61−66, Tartu, Vali Press.

Raudsepp, P., Roasto, M., Anton, D., Bleive, U., Kaldmäe, H., Meremäe, K., Kapp, K., Püssa, T., 2016. Overview of the objectives of the ERA-NET SUSFOOD international project "SUSMEATPRO": Sustainable plant ingredients for healthier meat products. Healthy Animal and Healthy Food, Estonian University of Life Sciences, Tartu, 97−100, Ecoprint.

Püssa, T., **Raudsepp, P**., Anton, D., Toomik, P., 2015. Stability of polyphenols in heat treatment of meat. Healthy Animal and Healthy Food, Estonian University of Life Sciences, Tartu, 122−128. Paar OÜ.

Raudsepp, P., 2014. Using plant extracts in food and cosmetics. Healthy Animal and Healthy Food, Estonian University of Life Sciences, Tartu, 83−91, Paar OÜ.

Anton, D., Roasto, M., Meremäe, K., Kuusik, S., **Raudsepp, P**., Laikoja, K., Elias, P., Püssa, T., 2013. Keefir, Kefirs, Kefyras, Кефир−the quality of the Baltic kefir products. Healthy Animal and Healthy Food, Estonian University of Life Sciences: 89−103, Paar OÜ.

5.2. Conference theses

Püssa, T., Frorip, A., Kuznetsov, A., Sünter, A., Anton, D., **Raudsepp, P**., 2018. New freshness index, method, and device to determine freshness status of meat and fish. DigICoMST website – http://icomst-proceedings.helsinki.fi/index.php: 64th International Congress of Meat Science and Technology (ICoMST 2018), Ed. Eero Puolanne. Helsinki, Finland: Helsinki University. Grand Hyatt, Melbourne, Australia, 12.−17.08. 2018.

Raudsepp, P., Libek, E., Rätsep, R., Kikas, A., Libek, A.-V., Arus, L., Kaldmäe, H., Bleive, U., 2017. Stability of anthocyanins and the variability of their content in some fruits and vegetables cultivated in Estonia. In: FoodBalt, Jelgava, Latvia, 27.−28.04. 2017.

Raudsepp, P., Anton, D., Roasto, M., Meremäe, K., Pedastsaar, P., Raal, A., Püssa, T., 2012. Kuue taimeleotise toimete võrdlus askorbiinhappe antioksüdantse ja klooramfenikooli ning naatriumnitriti antimikroobse toimega. Tervislik Toit: Healthy Animal and Healthy Food, Estonian University of Life Sciences, Tartu, 13−19, Pajoprint.

Roasto, M., Meremäe, K., **Raudsepp, P**., Anton, D., Pedastsaar, P., Mäesaar, M., Matt, D., 2012. The evaluation of antimicrobial properties of infusions and extracts of the plants grown in Estonia. FOODBALT-2012. 7-th Baltic Conference on Food Science and Technology, 1: FOODBALT-2012. Innovative and healthy food for consumers, 116−116, Ed. Petras Rimantas Venskutonis; Jonas Damašius. Kaunas University of Technology.

Raudsepp, P., Kaldmäe, H., Libek, A.-V., Kikas, A., 2009. Bioactive compounds and nutritional quality of berries in blackcurrant cultivars evaluated in Estonia. In: COST863 WG4 Joint Meeting with the 4th International Conference on Polyphenols and Health (ICPH2009) Bioactive compounds in berry fruits and human health benefits ABSTRACTS, 23−23. COST863 WG4 Joint Meeting with the 4th International Conference on Polyphenols and Health (ICPH2009). Harrogate International Centre, Harrogate, UK, 7.−10.12. 2009.

Püssa, T., Toomik, P., **Raudsepp, P**., Anton, D., 2009. Leukotoxin diols in mechanically deboned meat. Proceedings of World Congress on Oils and Fats & 28th ISF Congress, 9. Sydney, Australia, 27.−30.09. 2009.

Püssa, T., Vaher, M., Helmja, K., **Raudsepp, P**., Kaljurand, M., 2009. Methods of comparative analysis of the free radical scavenging

capability of components of antioxidant mixtures, oral presentation. Abstract Book and Program: 5th Conference of Nordic Separation Science Society, 56−56, Ed. Maria Borissova, Merike Vaher Tallinn Technical University Press, Tallinn, 26.−29.08. 2009.

Püssa, T., Anton, D, Pällin, R., **Raudsepp, P**., Toomik, P., Veri, K., Kirisaare, K., Rei, M., 2008. Berry polyphenols as inhibitors of fatty acid oxidation during marination of pork. Proceedings of 54th International Conference on meat science and technology, 3 pages. Cape Town, South Africa, 10.−15.08. 2008.

Raudsepp, P., Helmja, K., Raal, A., Vaher, M., Püssa, T., 2008. Comparative study of antioxidant capacity of *Rheum raponticum* root polyphenols. International conference on polyphenols, Salamanca, 8.−11.07. 2008.

Kaldmäe, H., **Raudsepp, P**., Kikas, A., Libek, A.-V., 2007. Focusing on nutritional quality of berries in breeding and introducing blackcurrant cultivars for Estonia. NJF Seminar 399 Beneficial health substances from berries and minor crops, Piikkiö, Finland, 3(1), 42. 14.−15.03. 2007.

Püssa, T., Pällin, R., Rei, M., **Raudsepp, P**., Soidla, R., 2006. Stability of plant polyphenol supplement during cooking and storage of mechanically deboned meat. Proceedings of 52th International Congress of Meat Science and Technology (52th ICoMST), 108−109, Ireland, Dublin, 13.−18.08. 2006.

6.3. Popular sciences papers

Moor, U., Põldma, P., Heinmaa, L., **Raudsepp, P**., 2016. Quality of apple juice depending on the type of apple production, storage conditions and method of pressing. Gardening Forum (2016), 16−20, Estonian Chamber of Agriculture and Commerce.

Raudsepp, P., 2015. Milline mahl on tervislik, Kodutohter 2015. 2.

Raudsepp, P., 2014. Taimeekstraktid leiavad tee nii kreemipurki kui toidulauale, Postimees, Maaelu edendaja, 9. 5. (12.05.2014).

Raudsepp, P., 2006. Valged mustikad ja valge põdrakanep" Eesti loodus. 2006. 2.

VIIS VIIMAST KAITSMIST

PRIIT KARIS
TOITUMUSE, LIPOMOBILISATISOONI JA INSULIINIRESISTENTSUSE SEOSED PIIMALEHMADEL
RELATIONSHIPS BETWEEN BODY CONDITION, LIPOMOBILIZATION AND INSULIIN RESISTANCE IN DAIRY COWS
Vanemteadur **Hanno Jaakson**, vanemteadur **Katri Ling**, kaasprofessor **Meelis Ots**
31. august 2021

HARES KHAN
KALTSIIDI AVAVEELINE SADENEMINE: PÕHJUSED JA TAGAJÄRJED GLOBAALSES JA KOHALIKUS VAATES
PELAGIC CALCITE PRECIPITATION IN LAKES: FROM A GLOBAL TO A LOCAL PERSPECTIVE ON ITS DRIVERS AND IMPLICATIONS
Doktor **Biel Obrador** (University of Barcelona, Hispaania), vanemteadur **Alo Laas**
16. september 2021

HEIKI LILL
ERINEVATE ENERGIA SALVESTUSTEHNOLOOGIATE UUDSED RAKENDUSPÕHIMÕTTED LIGINULLENERGIAHOONETES
NOVEL APPLICATION PRINCIPLES FOR ENERGY STORAGE TECHNOLOGIES IN NEARLY ZERO ENERGY BUILDINGS
Teadur **Alo Allik**, professor **Andres Annuk**
29. oktoober 2021

MARGUS ARAK
CULTIVATION TECHNOLOGY FOR LOW-BUSH BLUEBERRY CULTIVATION IN MILLED PEAT FIELD PLANTATIONS
AMMENDATUD FREESTURBAVÄLJADEL KASVATATAVA AHTALEHISE MUSTIKA MASINVILJELUSTEHNOLOOGIA
Professor **Jüri Olt**
1. november 2021

LIINA JÜRISOO
DISTRIBUTION OF AND DAMAGES BY DUTCH ELM DISEASE AGENTS IN NORTHEASTERN EUROPE
JALAKASURMA TEKITAJATE LEVIK JA KAHJUSTUSED KIRDE-EUROOPAS
Professor **Rein Drenkhan**
11. November 2021

ISSN 2382-7076
ISBN 978-9916-669-11-2 (trükis)
ISBN 978-9916-669-12-9 (pdf)